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Synthesis and Antibacterial Activity of *N,N*-Diethyl-3-substituted-2-(4-methyl-phenylsulfonamido)alkanamides and their Arylsulfonamide Precursors

Olayinka O. Ajani[1]*, Oluwole B. Familoni[2], Feipeng Wu[3], Johnbull O. Echeme[1] and Zheng Sujiang[4]

[1]*Department of Chemistry, Covenant University, Canaanland, P.M.B. 1023, Ota, Ogun State, Nigeria.*

[2]*Department of Chemistry, University of Lagos, Akoka, Lagos State, Nigeria.*

[3]*New Functional Polymeric Material Group, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (CAS), Beijing 100190, P.R. China.*

[4]*Test Center of Antimicrobial Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (CAS), Beijing 100190, P.R. China.*

ABSTRACT: A series of *N,N*-diethyl-3-substituted-2-(4-methylphenylsulfonamido)alkanamides **(8a-k)** and their arylsulfonamide precursors **(7a-k)** have been synthesized via facile approach. The chemical structures of the synthesized compounds were confirmed by analytical data and spectroscopic means. The *in vitro* antibacterial screening of these compounds along with streptomycin, showed *N,N*-diethyl-2-(4-methylphenylsulfonamido) -3-phenylpropanamide **(8j)** to be the most active agent on *Escherichia coli* at MIC of 12.5 µg/mL and on *Staphylococcus aureus* at MIC of 25 µg/mL.

KEYWORDS: in vitro stability; antibacterial activity; sulphonylation; substituted amide.

Introduction

Sulfonamide compounds were the first antimicrobial drugs which paved way for the unprecedented revolution in the world of antibiotics in medicine.[1] The first of such sulfonamides was actually a prodrug with the trade name Prontosil[1]. Since then, sulfonamides have continued to attract considerable attention in pharmaceutical development, because of their aqueous stability despite their net neutral charge[2]. Sulfonamides are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases[3].

The chemistry of sulfonamides has recently shown them to be highly efficient synthons in the preparation of various valuable biologically active compounds[4,5]. Although, many synthetic methods have been reported for the preparation of sulfonamides[6-8], but sulfonylation of ammonia[9] or primary and secondary amines with sulfonyl chlorides in the presence of a base is still being used as the method of choice because of high efficiency and simplicity of the reaction[10]. Sulfonamides are clinically important drugs in treating various gastrointestinal diseases and other forms of infections[11]. Apart from these, sulfonamides have also been reported to possess, among others, antibacterial[12,13], anti-HIV[14,15], antimalarial[16,17], antitumor[18,19], antiviral activity[20].

The mode of action of sulfonamide drug is based on the inhibition of DNA synthesis[21] by interfering with *para*-aminobenzoic acid (PABA) in biosynthesis of folic acid which is essential for growth of bacterial cells[22]. The sulfonamide group has been proven to have remarkable utility in medicinal chemistry and features in the structure of a number of clinically relevant small molecules[23]. For instance, some currently approved drugs with sulfonamide structural skeletons include; the antihypertensive agent *bosentan* **1**[24], the antiviral HIV protease inhibitor *amprenavir* **2**[25], the phophodiesterase-5 inhibitor *sildenafil* **3**[26], antidiabetic drug *glibenclamide* **4**[27], antidiabetic nonantibiotic *glimepiride* **5** and the diuretic drug *torasemide* **6**[28].

Epidemiological studies have revealed that emergence of new diseases is at the alarming rates in the recent time and that drug resistance is fasting becoming unbearable predicament in antimicrobial drug administration[29]. Methicillin-resistant staphylococci are resistant to many antibiotics such as penicillin, carbapenems, cephems and beta-lactam, quinolone, amino glycosides and tetracycline[30-32], whereas sulfonamides and their combination therapies are gaining more attention by the day in antimicrobial drug research[33]. Thus, it is conceivable to develop a series of functionalized sulfonamides with dialkylated amide side chain modification on carboxyl functionality for possible antibacterial efficacy.

* *For correspondence:* Olayinka O. Ajani,

Tel: +2348061670254

Email: ola.ajani@covenantuniversity.edu.ng

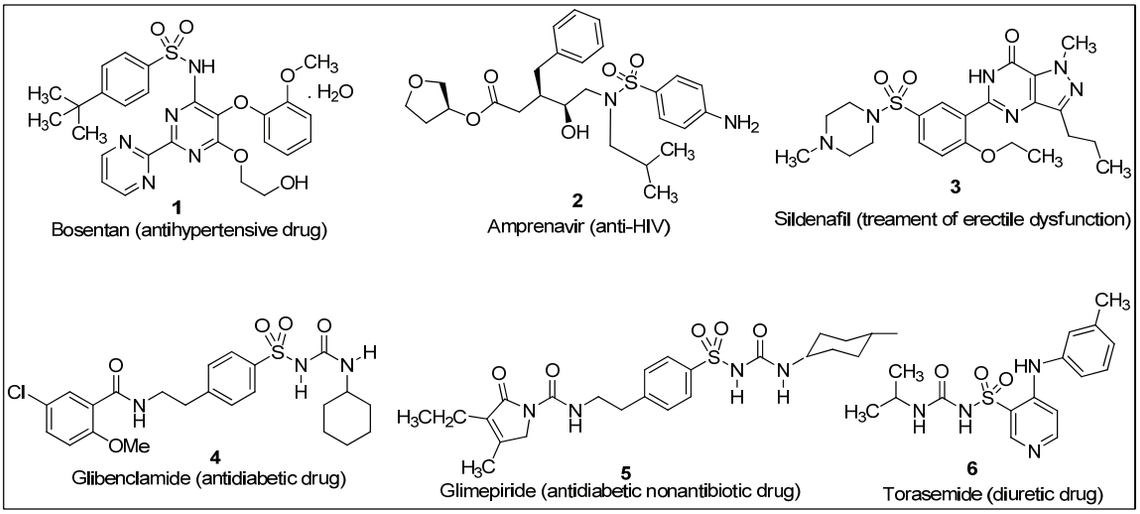


Fig. 1 Some important sulfonamide-based drugs available in market.

Results and Discussion

Chemistry

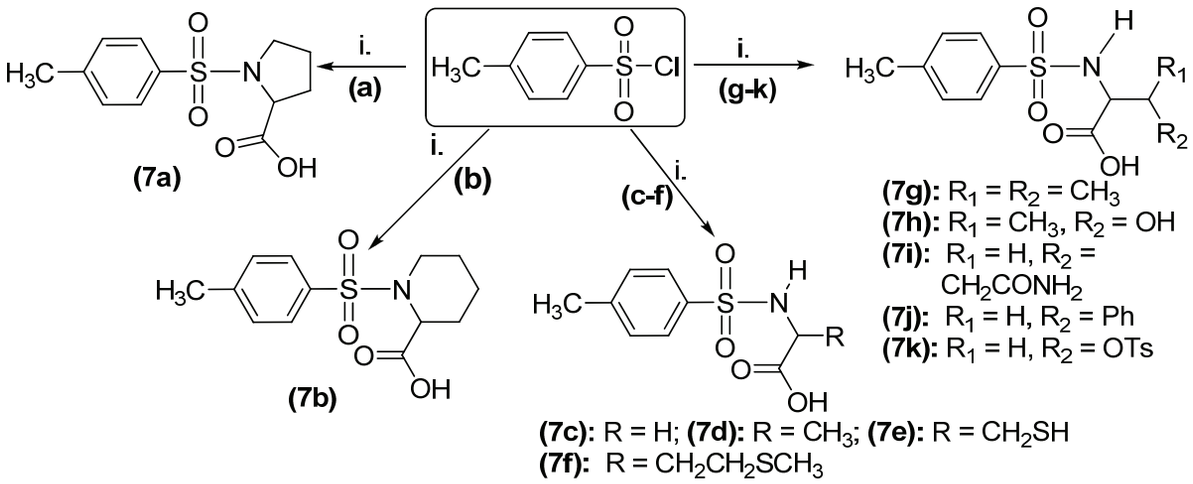
Arylsulfonamides of the type, *p*-toluenesulfonamides were successful prepared by condensation reaction of *p*-toluenesulfonyl chloride (*p*-TsCl) with various cheap and readily available amino acids in basified condition according to a known procedure with slight modification (Scheme 1). Although, some of these *p*-toluenesulfonamides have been reported before; however, slight modification of a known procedure[34] used in this study gave allowance for reaction augmentation and yield improvement. In addition, the *in vitro* antibacterial activity of these compounds on *Escherichia coli* and *Staphylococcus aureus* has not been investigated to our knowledge. In detail, the treatment of *p*-TsCl with equimolar quantity of 10 different amino acids afforded ten *p*-toluene sulfonamides **(7a-j)** while its reaction with half molar equivalent of tyrosine gave **(7k)** (Scheme 1). The coupling was achieved by adding *p*-TsCl to appropriate amino acid in aqueous sodium carbonate in three portions over a period of 1 h. The resulting solution was stirred at room temperature (RT) for 4 h and worked-up by acidification to pH 2.2 to afford *p*-toluenesulfonamides **(7a-k)** in good to excellent yields (60.5 – 99.0%).

Furthermore, formation of the amide bond has been one of the most widely studied reactions in organic chemistry because of its importance in biological systems. In view of this, we envisaged that incorporation of amide template within the sulfonamide scaffold may lead to increase in antibacterial activity. Another motivation behind synthesis of **(8a-k)** was based on the earlier report that disubstituted amides are more biologically active than the non-substituted counterparts[35]. Therefore, incorporation of amide group into the *p*-toulenesulfonamide was carried out in order to vary the antibacterial activity of such templates. This was achieved by the synthetic modification of the carboxyl side chain of the *p*-toluenesulfonamides **(7a-k)** via non-conventional amidation approach to afford *N,N*-diethyl substituted amido scaffolds **(8a-k)** (Table 1). Contrary to the conventional method of generating the acid chloride used in the earlier report[36], we have herein used Swern oxidation related approach which facilitated the synthesis of targeted *N,N*-diethyl amidated *p*-toluenesulfonamide in one pot two steps protocol[37]. This route does not require any purification step and the target compound precipitated from the reaction mixture, simplifying its handling and isolation in good yields.

According to spectral study, the structures of compounds (**7a-k)** as well as their *N,N*-diethyl amide bearing counterparts **(8a-k)** were deduced from their elemental analyses, as well as IR, high-field ^{1}H- and ^{13}C-NMR, and mass spectra. The results of the data were in agreement with the proposed structures. Synthetic conversion of *p*-toluenesulfonamide to *N,N*-diethyl amide derivatives was monitored spectroscopically using conversion of 3-methyl-2-(4-methylphenylsulfonamido) butanoic acid **(7g)** to *N,N*-diethyl-3-methyl-2-(4-methyl-phenylsulfonamido) butanamide **(8g)** as the representative template. The ^{1}H-NMR of **(7g)** showed two aromatic protons at δ 7.73-7.71 and 7.29-7.27 each while NH proton resonated as a doublet at δ 5.07-5.04. The signal at δ 2.41 was as a result of 3H of CH_3-Ph while the remaining two CH_3 protons resonated up-field as doublets at δ 0.97-0.96 and 0.88-0.86. On the other hand, **(8g)** showed three additional signals in its ^{1}H-NMR which were not present in **(7g)**. This signals were 4H multiplet at 3.15-3.02 (2 × $\underline{CH_2}$-CH_3), and two 3H triplet each at 1.03-1.01 ($\underline{CH_3}$-CH_2) and 0.84-0.81 ($\underline{CH_3}$-CH_2). This implies the presence of

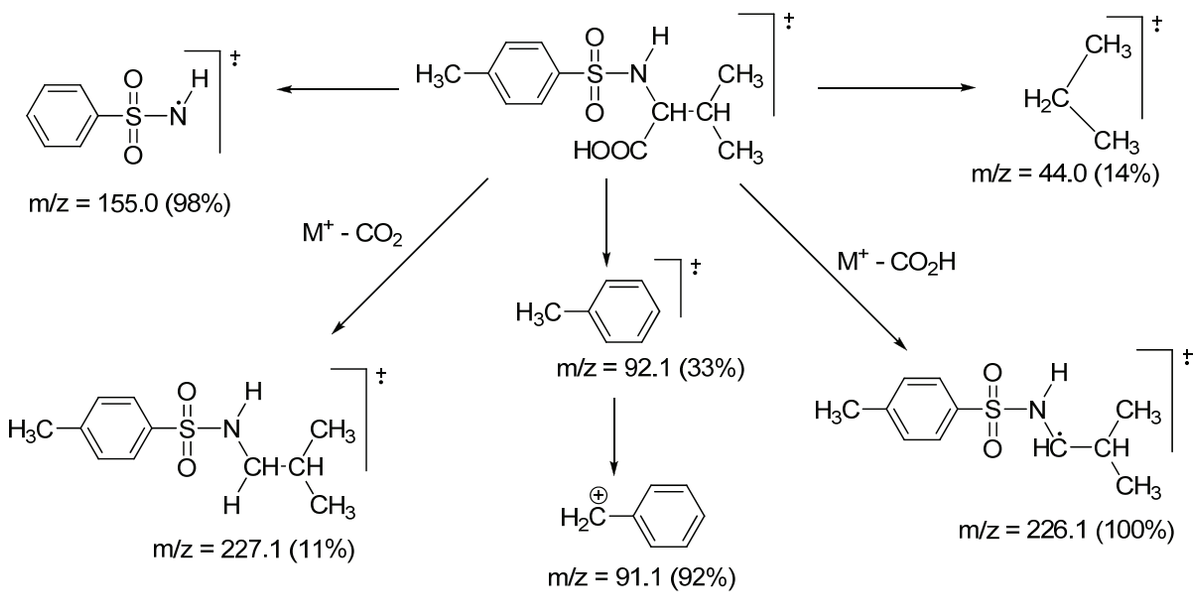
$(CH_3CH_2)_2N$-(C=O) as structural feature in **(8g)** which signified effective amidation of **(7a)**.

In like manner, the infrared stretching vibration band of N-H was observed at 3289 cm^{-1} and 3260 cm^{-1} for **(7g)** and **(8g)** respectively whereas the band at 1595 cm^{-1} depicted the presence of C=C of aromatic. The C=O of acid in **(7g)** appeared at 1711 cm^{-1} whereas, the same band shifted to 1690 cm^{-1} in the compound **(8g)**, indicating the conversion of acid functionality to amide group. The mass spectrum of **(7g)** showed peaks at m/z of 227.1 which represented loss of CO_2 while its base peak at m/z 226.1 was as a result of loss of COOH. Its mass spectrum did not show molecular ion peak, probably because there could have been fragmentation before the molecule reached the detector. The mass spectrum of **(7g)** was also characterized by the occurrence of some daughter fragments at m/z 155.0, 92.1, 91.1, 65.0 with relative intensities of 98%, 33%, 92% and 48% respectively (Scheme 2).



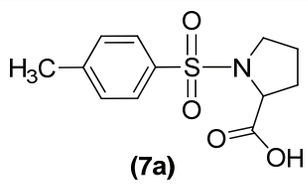
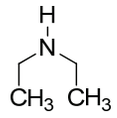
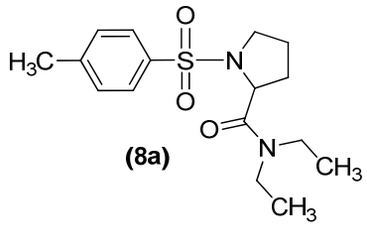
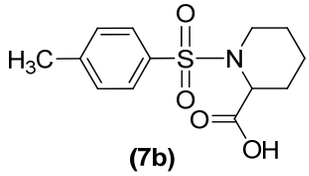
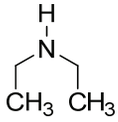
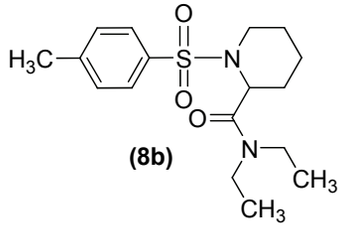
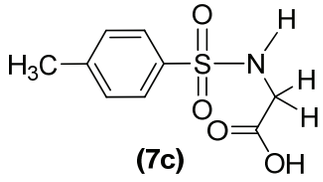
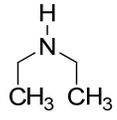
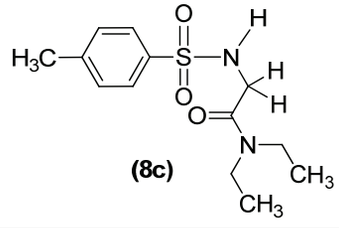
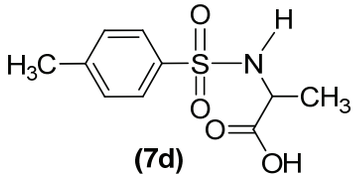
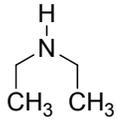
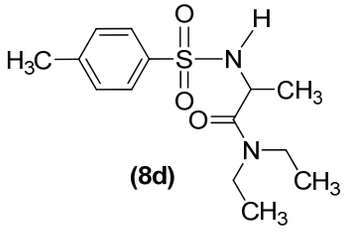
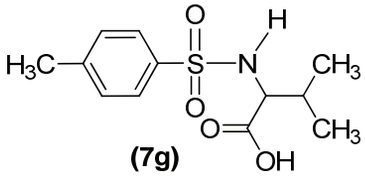
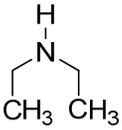
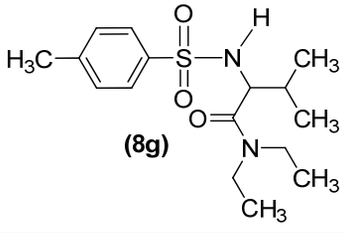
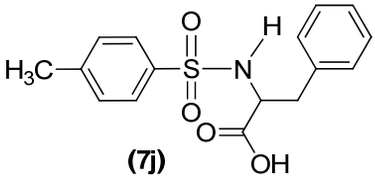
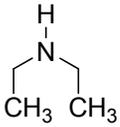
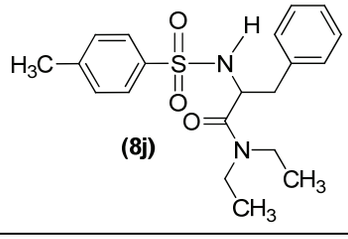
Reaction Conditions: i. Na_2CO_3, H_2O, 0 °C to rt, 4 h, 2 N HCl. **(a)** L-Proline **(b)** L-Pipecolic acid **(c)** Glycine **(d)** L-Alanine **(e)** L-Cysteine **(f) L-**Methionine **(g)** L-Valine **(h)** L-Threonine **(i)** L-Glutamine **(j) L-**Phenyl alanine **(k)** L-Tyrosine.

Scheme 1 Pathway for the synthesis of arylsulfonamide precursors **(7a-k).**



Scheme 2 Proposed fragmentation pattern for mass spectrum of **(7g).**

Table 1 Conversion of p-toluenesulfonamide to *N,N*-diethylamido sulfonamide products.

Entry	*p*-Toluenesulfonamide	Amidating Agent	*N,N*-diethyl amide product
1	 (7a)		 (8a)
2	 (7b)		 (8b)
3	 (7c)		 (8c)
4	 (7d)		 (8d)
5	 (7g)		 (8g)
6	 (7j)		 (8j)

Biological Evaluation

The antimicrobial sensitivity testing of the nineteen synthesized compounds were assayed using agar diffusion technique[38]. For the sake of brevity and better understanding, the selectivity index of the synthesized *p*-tolylsulfonamide derivatives on *E. coli* is as shown in Fig. 2. The selectivity index, which was evaluated by comparing the zone of inhibition (mm) obtained from each of the synthesized

compounds with that of clinical standard (streptomycin), gave a clearer picture of the antibacterial activity of this group of sulfonamides on the targeted organisms. Although, majority of the *p*-tolylsulfonamides have moderate to high activity, but none of them could compete with the streptomycin in *E. coli* growth inhibition efficacy. It was observable that the S.I. of *p*-tolylsulfonamide varied from 0.5 for **(7c)** to 0.98 for **(8j)**. Unequivocally speaking, streptomycin, with S.I. value of unity, demonstrated high level of superiority to all the synthesized *p*-tolylsulfonamide on the inhibition of *E. coli* growth. The highest activity was observed in **(8j)** with S.I. of 0.98. This improved activity might be as a result of additional conjugation which occurred in **(8j)**, other compounds that showed high activity include **(7d)**, **(7e)**, **(7k)**, **(8a)**, **(8b)**, **(8c)**, **(8d)** and **(8g)** (S.I. > 0.8). Within the class, it was noticeable that only seven compounds showed moderate activity, in a decreasing order **(7f)** ≈ **(7g)** > **(7h)** > **(7j)** > **(7a)** ≈ **(7i)** > **(7b)** (0.6 < S.I. < 0.8) while the least activity which was categorized by S.I. < 0.6, was experienced in one compound **(7c)** with S.I. value of 0.5 to be precised.

In like manner, the antibacterial activity of *p*-tolylsulfonamides with respect to streptomycin antibiotic on the *S. aureus* clinical isolate was also evaluated and pictorially presented as shown in Fig. 3. Based on the intensity of the selectivity index, **(8j)** could be considered as the most active (S.I. value = 1.6) while **(7a)** and **(8g)** were the least active having S.I. value of 0.47. It is worthy to note that two compounds **(7f)** and **(8j)** were more active than the streptomycin as far as *S. aureus* screening was concerned. All other *p*-tolylsulfonamides were less active than streptomycin except **(7i),** which in this case, competed favourably with the streptomycin standard. Hence, ten *p*-tolylsulfonamides exhibited moderate activity on *S. aureus* in a decreasing order as **(7k)** > **(7g)** > **(7d)** ≈ **(7e)** ≈ **(7h)** ≈ **(8a)** > **(7j)** > **(8c)** ≈ **(8b)** > **(8d)** (0.5 < S.I. < 0.8) whereas lesser activity was exhibited by compounds **(7c)** > **(7b)** > **(7a)** ≈ **(8g)** (S.I. < 0.5).

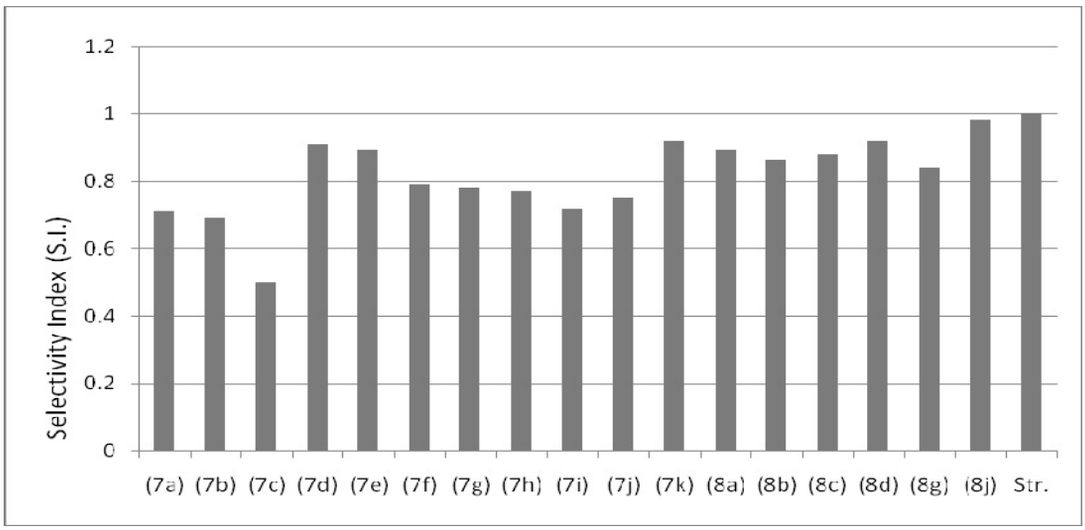


Fig. 2 Antibacterial activity of the synthesized compounds against *Escherichia coli.*

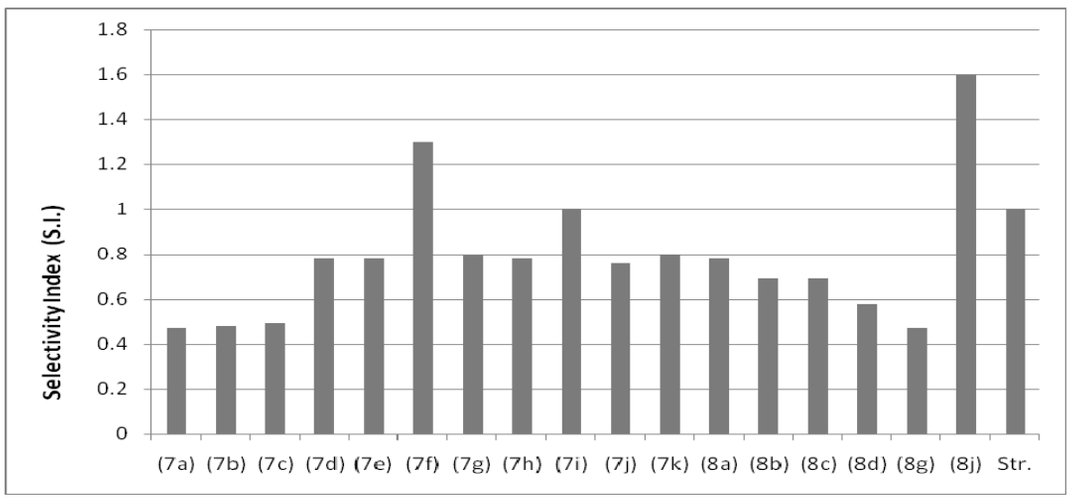


Fig. 3 Antibacterial activity of *p*-tolylsulfonamides against *Staphylococcus aureus.*

Furthermore, in order to establish the lowest concentration at which the growth of *E. coli* and *S. aureus* was inhibited by *p*-tolylsulfonamide scaffolds, the MIC test was carried out according to a standard procedure[39] and the result is as shown in Table 2. From the point of view of *E. coli,* the MIC values ranged from 12.5 µg/mL **(8j)** to 1000 µg/mL **(7c)** while the MIC value for streptomycin under similar condition was 6.25 µg/mL. Although, streptomycin was more active than any of the *p*-tolylsulfonamides, it was apparent that different *p*-tolylsulfonamides exhibited varying MIC values against the strain of *E. coli.* Thus, the MIC values ranging from 12.5 µg/mL to 50 µg/mL was observable among **(8j)**, (**8d)**, (**7k)**, (**7d)**, (**7e)**, (**8a)** and (**8c)**; between 62.5 µg/mL and 100 µg/mL for (**8b)**, (**8g)**, (**7f)** and (**7g)**; between 125 µg/mL and 500 µg/mL for **(7a), (7b), (7i), (7h)** and **(7j)** and the least activity was observed in compound **(7c)** with an MIC value of 1000 µg/mL.

In addition, the MIC test for the series of *p*-tolylsulfonamides was carried out on *S. aureus* (Table 2) and it should be noted that the lowest MIC value culminated into highest potency[40]. It is therefore paramount to note that the highest potency was observed in **(8j)** (MIC = 25 µg/mL) while compound **(8g)** exhibited the least potency (MIC = 1000 µg/mL) on *S. aureus*. This is higher activity than the one reported by Ghorab *et al.,* (2004). From comparative study, many members of this group such as **(7d) - (7k), (8a)** and **(8j)** (MIC = 25 µg/mL – 100 µg/mL) were more active than streptomycin (MIC = 125 µg/mL) using the MIC test involving *S. aureus*. Under the same condition, **(8b)** (MIC = 125 µg/mL) competed favorably with streptomycin in its inhibitory potential on *S. aureus*. Seven compounds **(7d)**, **(7e)**, **(7g)**, **(7h)**, **(7j)**, **(7k)** and **(8a)** had MIC value 100 µg/mL and other seven *p*-tolylsulfonamides **(8b)**, **(8c)**, **(8d)**, **(7a)**, **(7b)**, **(7c)** and **(8g)** had MIC values between 125 µg/mL and 1000 µg/mL.

Furthermore, from the structure activity relationship (SAR) study, it was observed that the nature of side chains (R and R_1-CH-R_2) of the *p*-tolylsulfonamides (Scheme 1) and the presence of *N,N*-diethylated amido moieties $\{(CH_3CH_2)_2N\text{-}C{=}O\}$ of the amide (Table 1) contributed immensely toward synergistic or antagonistic effect on the reported *in vitro* antibacterial activity. For instance, regarding the trend of activity against *E. coli*, **(8a)** and **(8g)** were two times more active **(7a)** and **(7g)** respectively while **(8b)** was four times more active than **(7b)**. In fact, **(8c)** was twenty times more active than **(7c)**. The compounds **(8a-8c)**, **(8g)** were structurally related with **(7a-7c)**, **(7g)**, but the only disparity was the presence of *N,N*-diethyl amide chain in the former, which probably accounted for their better activities than the latter.

In view of these, there was a clear indication that presence of *N,N*-disubstituted amide led to an upward trend in the activity against *E. coli.* This was in agreement with earlier report that *N,N*-disubstituted thiosemicarbazones are more active than their mono- and non-substituted counterparts on *S. aureus* and *E. coli*[35]. Considering the effect of side chain, **(8j)** (R = Ph-CH_2) was four times more active than the **(8d)** (R = H-CH_2) on *E. coli*. This was as a result of π-π stack character introduced by the Ph group which is weakly activating in nature and the extensive conjugation contributed by C=C aromatic from the Ph in the case of **(8j)**. In a similar manner, **(8j)** was ten times more active than **(8d)** on *S. aureus*. Synergistic contribution of *N,N*-diethyl amide caused **(8b)** and **(8c)** to be two times more active than **(7b)** and **(7c)** respectively on *S. aureus.*

Table 2 MIC test of *p*-tolylsulfonamides on targeted organisms (µg/mL).

Organisms→ Compd. No↓	*E. coli* ATCC 25922		*S. aureus* ATCC 6538	
	@100 µg/mL	@1000 µg/mL	@100 µg/mL	@1000 µg/mL
(7a)	>100	125	>100	250
(7b)	>100	250	>100	250
(7c)	>100	1000	>100	500
(7d)	25	<1000	100	<1000
(7e)	50	<1000	100	<1000
(7f)	100	<1000	50	<1000
(7g)	100	<1000	100	<1000
(7h)	>100	500	100	<1000
(7i)	>100	250	62.5	<1000
(7j)	>100	500	100	<1000
(7k)	25	<1000	100	<1000
(8a)	50	<1000	100	<1000
(8b)	62.5	<1000	>100	125
(8c)	50	<1000	>100	250
(8d)	25	<1000	>100	250
(8g)	62.5	<1000	>100	1000
(8j)	12.5	<1000	25	<1000
Str.	6.25	<1000	>100	125

>100 means that if there was no growth inhibition at 100 µg/mL, it was repeated at 1000 µg/mL, <1000 µg/mL means that growth inhibition has already been experienced at lower concentration less than or equal to 100 µg/mL; hence, there is no need to repeat the test at 1000 µg/mL. – means no activity was observed even at 1000 µg/mL. **Str.** means Streptomycin clinical reference.

Conclusion

Sulfonamide drugs which have brought about an antibiotic revolution in medicine are associated with a wide range of

biological activities. *N,N*-Diethylsubstituted amide bearing sulfonamides were herein successfully synthesized from *p*-toluenesulfonamide precursors via non-conventional amidation approach. The antibacterial activity of the synthesized compounds was evaluated upon *Escherichia coli* and *Staphylococcus aureus* as the targeted organisms. *N,N*-Diethyl-2-(4-methylphenylsulfonamido)-3-phenylpropanamide **(8j)** emerged as the most active antibacterial agents with MIC values of 12.5 µg/mL and 25 µg/mL on *E. coli* and *S. aureus* respectively.

Experimental

General

Melting points were determined on XT-4 digital microscopic melting point apparatus and were uncorrected. Infra red spectra were recorded as KBr disc on Varian Excalibur HE 3100 FT-IR Spectrometer while mass spectra were obtained using Waters GCT Premier Spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6, unless otherwise stated, on NMR Bruker DPX 400 spectrometer operating at 400 MHz and 100 MHz respectively (δ in ppm relative to Me_4Si). The elemental analysis (C, H, N) of the synthesized compounds were performed using a Flash EA 1112 elemental analyzer. In addition, the pH was monitored and confirmed during acidification with a Portable pH Meter Model PHB4. All drying were conducted at reduced pressure with DHG-9023A Vacuum Oven. Results were found to be in good agreement with the calculated values (Table 1). All compounds were routinely checked by TLC on silica gel G plates and column chromatographic purifications were carried out on Merck silica gel F (Mesh 200-300) using chloroform/ methanol (9:1, v/v) solvent system and the developed plates were visualized under UV light. All the amino acids used were obtained from Aladdin Chemical Co. Ltd. while *p*-toluenesulfonyl chloride was supplied by Huaxueshiji China. All other chemicals were obtained from Beijing Chemical Works, China. Solvents used were of analytical grade and, when necessary, were purified and dried by standard methods.

General procedure for synthesis of p-toluenesulfonamides (7a-k)

Na_2CO_3 (2.785 g, 26.25 mmol) was added to a solution of amino acid (12.5 mmol) in H_2O (15 mL) at 0 °C followed by addition of *p*-toluenesulfonyl chloride, *p*-TsCl (2.86 g, 15 mmol) in three portions over a period of 1 h. The slurry was then warmed to room temperature and allowed to stir for 4 h. Upon completion of the reaction which was TLC monitored using $CHCl_3/CH_3OH$ solvent system (9:1), the reaction mixture was acidified with 20 % concentrated aqueous HCl solution to pH 2, after which crystallization occurred and the product was obtained via suction filtration. The filtered crude product was washed with pH 2.2 buffer and dried in a vacuum oven at 60 °C for 12 h to afford *p*-toluenesulfonamides **(7a-k)** in good to excellent yields (60.5 – 99.0%).

1-Tosylpyrrolidine-2-carboxylic acid (7a)

Yield 3.23 g (95.9%), mp 41-43 °C, {Lit. mp 42-44 °C},[34] R_f = 0.82 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 7.72-7.70 (d, *J* = 8 Hz, 2H, Ar-H), 7.43-7.41 (d, *J* = 8 Hz, 2H, Ar-H), 4.08-4.05 (m, 1H, CH-COOH), 3.36-3.30 (m, 1H, CHb of CH_2-N), 3.16-3.10 (m, 1H, CHa of CH_2-N), 2.39 (s, 3H, CH_3), 1.89-1.77 (m, 3H, CH & CH_2), 1.56-1.50 (m, 1H, CH). ^{13}C-NMR (DMSO-d_6) δ: 173.2 (CO), 143.5, 134.7, 129.9, 127.2, 67.1, 60.5, 48.5, 30.5, 25.2, 24.3, 21.1. IR (KBr) cm^{-1}: 3217 (OH), 2939 (CH aromatic), 2860 (CH aliphatic), 1734 (C=O of COOH), 1601 (C=C aromatic), 1184, 1151 (SO_2 two bands), 662 (Ar-H).

1-Tosylpiperidine-2-carboxylic acid (7b)

Yield 3.36 g (95%), R_f = 0.89 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR ($CDCl_3$) δ: 7.71-7.63 (d, *J* = 8 Hz, 2H, Ar-H), 7.29-7.24 (d, *J* = 8 Hz, 2H, Ar-H), 4.74-4.73 (m, 1H, CH-COOH), 3.72-3.69 (dd, J_1 = 10.4 Hz, J_2 = 20 Hz, 1H), 3.23-3.18 (dd, J_1 = 12 Hz, J_2 = 20 Hz, 1H), 2.41 (s, 3H, CH_3), 2.18-2.15 (dd, J_1 = 3.7 Hz, J_2 = 12.7 Hz, 1H), 1.74-1.55 (m, 4H), 1.48-1.35 (m, 3H, CH_2 & CH). ^{13}C-NMR (DMSO-d_6) δ: 171.8 (CO), 142.9, 137.4, 129.6, 129.4, 126.9, 126.8, 54.6 (CH-CO), 42.1 (CH_2-N), 27.0, 23.9, 19.7 (CH_2), 21.0 (CH_3-Ph). MS: in m/z [rel. %]: 239.1 [M^+ - CO_2, 10%], 238.1 [M^+ - COOH, 74%], 220.1 [37%], 191.1 [M^+ - $PhCH_2$, 28%], 91.1 [$PhCH_2^+$, 100%]. Anal. calcd. for $C_{13}H_{17}NO_4S$ (283.35): C, 55.11; H, 6.05; N, 4.94. Found: C, 54.10; H, 6.08; N, 5.02.

2-(4-Methylphenylsulfonamido)acetic acid (7c)

Yield 2.75 g (95.8%), mp 145-146 °C, {Lit. mp 147 °C},[41] R_f = 0.47 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 12.63 (s-br, 1H, OH of -COOH), 7.95-7.92 (t, *J* = 6 Hz, 1H, NH-CH_2), 7.68-7.66 (d, *J* = 8 Hz, 2H, Ar-H), 7.38-7.36 (d, *J* = 8 Hz, 2H, Ar-H), 3.55-3.53 (d, *J* = 6 Hz, 2H, CH_2-NH), 2.37 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3279 (N-H), 3102 (CH aromatic), 2980 (CH aliphatic), 1726 (C=O of COOH), 1595 (C=C aromatic), 1234, 1161 (SO_2 two bands), 669 (Ar-H). MS: in m/z [rel. %]: 238.1 [41%], 184.0 [55%], 155.0 [$PhCH_2SO_2^+$, 100%], 91.1 [$PhCH_2^+$, 65%], 65.0 [63%].

2-(4-Methylphenylsulfonamido)propanoic acid (7d)

Yield 2.51 g (82.6%), mp 116-118 °C, R_f = 0.78 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 8.05-8.03 (d, *J* = 8.3 Hz, 1H, NH), 7.68-7.65 (d, *J* = 8 Hz, 2H, Ar-H), 7.37-7.35 (d, *J* = 8 Hz, 2H, Ar-H), 3.74-3.71 (m, 1H, CH), 2.37 (s, 3H, CH_3-Ph), 1.13-1.12 (d, *J* = 7.2 Hz, 3H, CH_3-CH). IR (KBr) cm^{-1}: 3277 (N-H), 3084 (OH), 2934 (CH aliphatic), 1715 (C=O of COOH), 1595 (C=C

aromatic), 1233, 1150 (SO_2 two bands), 677 (Ar-H). MS: in m/z [rel. %]: 199.1 [M^+ - CO_2, 12%], 198.0 [M^+ - CO_2H, 89%], 156.0 [$CH_3PhSO_2^+$, 21%], 155.0 [$PhCH_2SO_2^+$, 97%], 91.1 [$PhCH_2^+$, 100%], 65.0 [47%], 44.1 [27%].

3-Mercapto-2-(4-methylphenylsulfonamido) propanoic acid (7e)

Yield 3.03 g (88.1%), mp 161-164 °C, R_f = 0.39 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 8.59-8.53 (s-br, 1H, SH), 8.29-8.27 (d, *J* = 8.4 Hz, 1H, NH-CH), 7.71-7.69 (m, 1H, CH_2-CH-NH), 7.65-7.58 (d, *J* = 8 Hz, 2H, Ar-H), 7.48-7.46 (d, *J* = 7.6 Hz, 1H, CH_b of CH_2), 7.35-7.33 (d, *J* = 8 Hz, 2H, Ar-H), 7.12-7.10 (d, *J* = 7.6 Hz, 1H, CH_a of CH_2), 2.37 (s, 3H, CH_3-Ph). IR (KBr) cm^{-1}: 3445 (N-H), 3003 (CH aromatic), 2907 (CH aliphatic), 1736 (C=O of COOH), 1596 (C=C aromatic), 1221, 1152 (SO_2 two bands), 679 (Ar-H).

2-(4-Methylphenylsulfonamido)-4-(methylthio) butanoic acid (7f)

Yield 2.62 g (69.1%), mp 105-106 °C, R_f = 0.80 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR ($CDCl_3$) δ: 7.75-7.73 (d, *J* = 8 Hz, 2H, Ar-H), 7.29-7.27 (d, *J* = 8 Hz, 2H, Ar-H), 4.07 (s-br, 1H, NH), 2.50-2.48 (m, 1H, CH-NH), 2.45-2.33 (m, 2H, CH_2-S), 2.40 (s, 3H, CH_3-Ph), 2.10-2.03 (m, 1H, CH of CH_2), 1.99 (s, 3H, CH_3-S), 1.94-1.87 (m, 1H, CH of CH_2). IR (KBr) cm^{-1}: 2997 (CH aromatic), 2911 (CH aliphatic), 1726 (C=O of COOH), 1591 (C=C aromatic), 1220, 1144 (SO_2 two bands), 696 (Ar-H).

3-Methyl-2-(4-methylphenylsulfonamido) butanoic acid (7g).

Yield 3.21 g (94.7%), mp 125-126 °C, R_f = 0.81 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR ($CDCl_3$) δ: 7.73-7.71 (d, *J* = 8 Hz, 2H, Ar-H), 7.29-7.27 (d, *J* = 8 Hz, 2H, Ar-H), 5.07-5.04 (d, *J* = 9.9 Hz, 1H, NH), 3.82-3.78 (dd, J_1 = 4.6 Hz, J_2 = 9.9 Hz, 1H, CH-CH-NH), 2.41 (s, 3H, CH_3-Ph), 2.13-2.08 (m, 1H, CH), 0.97-0.96 (d, *J* = 6.8 Hz, 3H, CH_3), 0.88-0.86 (d, *J* = 6.8 Hz, 3H, CH_3). IR (KBr) cm^{-1}: 3289 (N-H), 2967 (CH aromatic), 2876 (CH aliphatic), 1711 (C=O of COOH), 1595 (C=C aromatic), 1335, 1163 (SO_2 two bands), 687 (Ar-H). MS: in m/z [rel. %]: 227.1 [M^+ - CO_2, 11%], 226.1 [M^+ - CO_2H, 100%], 155.0 [$PhCH_2SO_2^+$, 98%], 92.1 [$PhCH_3^+$, 33%], 91.1 [$PhCH_2^+$, 92%], 65.0 [48%], 44.0 [$CH_2(CH_3)_2^+$, 14%]. Anal. calcd. for $C_{12}H_{17}NO_4S$ (271.34): C, 53.12; H, 6.32; N, 5.16. Found: C, 52.91; H, 6.33; N, 5.08.

3-Hydroxy-2-(4-methylphenylsulfonamido) butanoic acid (7h).

Yield 3.22 g (94.4%), mp 90-92 °C, R_f = 0.62 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 7.68-7.66 (d, *J* = 8 Hz, 2H, Ar-H), 7.52-7.50 (d, *J* = 9.2 Hz, 1H, NH-CH), 7.34-7.32 (d, *J* = 8 Hz, 2H, Ar-H), 3.95-3.91 (m, 1H, CH), 3.64-3.61 (dd, J_1 = 3.6 Hz, J_2 = 9.2 Hz, 1H, CH-CH-NH), 2.36 (s, 3H, CH_3-Ph), 2.08 (s, 1H, -OH), 1.01-0.99 (d, *J* = 6.36 Hz, 3H, CH_3-CH). IR (KBr) cm^{-1}: 3501 (OH free), 3435 (N-H), 3360 (OH of COOH), 3262 (N-H), 2976 (CH aliphatic), 1697 (C=O), 1595 (C=C aromatic), 1167 (SO_2), 673 (Ar-H).

5-Amino-2-(4-methylphenylsulfonamido)-5-oxopentanoic acid (7i).

Yield 3.10 g (82.7%), mp 145-146 °C, R_f = 0.14 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR (DMSO-d_6) δ: 8.06-8.04 (d, *J* = 8.76 Hz, 1H, NH-CH), 7.65-7.63 (d, *J* = 8 Hz, 2H, Ar-H), 7.36-7.34 (d, *J* = 8 Hz, 2H, Ar-H), 7.24 (s, 1H, NH of NH_2), 6.73 (s, 1H, NH of NH_2), 3.69-3.65 (dd, J_1 = 8.76 Hz, J_2 = 17.24 Hz, 1H, CH-COOH), 2.37 (s, 3H, CH_3-Ph), 2.08-2.04 (t, *J* = 7.68 Hz, 2H, CH_2), 1.83-1.80 (t, *J* = 4 Hz, 1H, CH), 1.67-1.61 (q, J_1 = 4 Hz, J_2 = 7.6 Hz, CH). IR (KBr) cm^{-1}: 3456 (N-H), 3331 (OH of COOH), 3246 (N-H), 2955 (CH aliphatic), 1678 (C=O), 1640 (C=O), 1570 (C=C aromatic), 1321, 1167 (SO_2 two bands), 685 (Ar-H). MS: in m/z [rel. %]: 246.0 [97%], 238.1 [16%], 171.0 [$CH_3PhSO_2NH_2^+$, 49%], 156.0 [$CH_3PhSO_2^+$, 84%], 139.0 [52%], 123.0 [100%], 92.1 [$PhCH_3^+$, 38%], 44.0 [$CONH_2^+$, 32%]. Anal. calcd. for $C_{12}H_{16}N_2O_5S$ (300.34): C, 47.99; H, 5.37; N, 9.33. Found: C, 47.77; H, 5.39; N, 9.04.

2-(4-Methylphenylsulfonamido)-3-phenylpropanoic acid (7j)

Yield 3.95 g (99.0%), 159-160 °C {Lit. mp 161 °C},[41] R_f = 0.76 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR ($CDCl_3$) δ: 7.61-7.59 (d, *J* = 8 Hz, 2H, Ar-H), 7.24-7.21 (m, 5H, Ar-H), 7.10-7.08 (d, *J* = 8 Hz, 2H, Ar-H), 5.16-5.14 (d, *J* = 8.64 Hz, 1H, NH), 4.22-4.17 (ddd, J_1 = 5.6 Hz, J_2 = 6.4 Hz, J_3 = 8.64 Hz, 1H, CH-COOH), 3.12-3.08 (dd, J_1 = 5.6 Hz, J_2 = 20 Hz, 1H), 3.03-2.98 (dd, J_1 = 6.4 Hz, J_2 = 20 Hz, 1H), 2.40 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 175 (CO), 143.9, 136.6, 134.9, 129.8 (2CH aromatic), 129.6 (2CH aromatic), 128.8 (2CH aromatic), 127.5, 127.2 (2CH aromatic), 56.5, 39.0 (CH_2), 21.7 (CH_3). IR (KBr) cm^{-1}: 3350 (N-H), 3188 (OH), 3057 (CH aromatic), 2961 (CH aliphatic), 1736 (C=O of COOH), 1350, 1171 (SO_2 two bands), 675 (Ar-H).

2-(4-Methylphenylsulfonamido)-3-(4-(tosyloxy)phenyl) propanoic acid (7k)

Yield 3.70 g (60.5%), mp 101-103 °C, R_f = 0.76 ($CHCl_3/CH_3OH$, 9:1, at RT). ^{1}H-NMR ($CDCl_3$) δ: 7.69-7.67 (d, *J* = 8.26 Hz, 2H, Ar-H of OTs), 7.59-7.57 (d, *J* = 8.26 Hz, 2H, Ar-H of OTs), 7.32-7.30 (d, *J* = 8 Hz, 2H, Ar-H), 7.24-7.22 (d, *J* = 8 Hz, 2H, Ar-H), 7.03-7.01 (d, *J* = 8.4 Hz, 2H, Ar-H of benzyl), 6.85-6.83 (d, *J* = 8.4 Hz, 2H, Ar-H of benzyl), 5.14-5.12 (d, *J* = 8.5 Hz, 1H, NH-CH), 4.17-4.12 (q, *J* = 6.8 Hz, 1H, NH-CH-CH_2), 3.11-3.06 (dd, J_1 = 5.2 Hz, J_2 = 20 Hz, 1H, CH of CH_2-Ar), 2.97-2.92 (dd, J_1 = 6.8 Hz, J_2 = 20 Hz, 1H, CH of CH_2-Ar), 2.45 (s, 3H, CH_3-OTs), 2.41 (s, 3H, CH_3-Ph). ^{13}C-NMR ($CDCl_3$) δ: 173.9 (CO), 148.9, 145.7, 144.2, 136.4, 134.3, 132.4 (six benzylic

aromatic carbon atoms), 130.9, 130.9, 130.0, 130.0, 129.9, 129.9, 128.6, 128.6, 127.2, 127.2, 122.6, 122.6 (twelve sulfonyl aromatic carbon atoms), 56.4 (CH), 38.3 (benzylic CH_2), 21.9 (CH_3 linked to SO_3-Ar), 21.7 (CH_3 linked to SO_2-Ar). IR (KBr) cm^{-1}: 3561 (N-H), 3339 (OH of COOH), 2924 (CH aliphatic), 1717 (C=O), 1559 (C=C aromatic), 1150, 1092 (SO_2 two bands), 669 (Ar-H). MS: in m/z [rel. %]: 443.1 [M^+ - COOH, 23%], 171.0 [$CH_3PhSO_2NH_2^+$, 35%], 156.0 [$CH_3PhSO_2^+$, 58%], 155.0 [$PhCH_2SO_2^+$, 90%], 134.1 [90%], 92.1 [$PhCH_3^+$, 64%], 65.0 [100%]. Anal. calcd. for $C_{23}H_{23}NO_7S_2$ (489.57): C, 56.43; H, 4.74; N, 2.86. Found: C, 54.16; H, 4.79; N, 5.31.

General procedure for N,N-diethylalkanamide of p-toluenesulfonamide (8a-k)

A three-necked 250 mL flask equipped with magnetic stirring bar was charged with appropriate *p*-toluenesulfonamide **(7a-k)** (2.96 mmol) and dichloromethane (DCM) (10 mL). The flask was stoppered, cooled to 0 °C and N_2 was bubbled into it continuously. Oxalyl chloride (0.34 mL, 3.85 mmol, 1.3 equiv.) was added via dropping pipette to maintain the temperature below 10 °C followed by the addition of 2 drops of dimethyl formamide (DMF). The resulting mixture was stirred at room temperature until the conversion to acid chloride was completed (i.e. for about 1.5 h) and then concentrated to dryness with rotary evaporator (23 °C, 40 mmHg). Dichloromethane (DCM) (20 mL) was added to the resulting crude acid chloride and the solution was concentrated again.

In a separate 250 mL three-necked round bottom flask, equipped with a magnetic stirring bar, a N_2 inlet, a rubber septum, 125-mL pressure equalizing addition funnel and a temperature probe was charged with dichloromethane (DCM) (10 mL), triethylamine (0.62 mL, 4.44 mmol, 1.5 equiv.) and diethylamine (0.4 mL, 3.85 mmol, 1.3 equiv.) and the mixture was cooled to -10 °C (acetone/ice bath). The crude acid chloride was dissolved in dichloromethane (DCM) (10 mL) and this solution was transferred to the addition funnel. The acid chloride was then added dropwisely to the stirred diethylamine solution at such a rate that the internal temperature was maintained below 10 °C. Upon completion of the addition of the acid chloride solution (ca 30 min), the mixture was stirred at -10 to 0 °C for 1 h and at room temperature for 1 h.

The mixture was then diluted with 2M HCl (6 mL) and was transferred into a 250 mL separatory funnel and the layers separated. The organic layer was washed with brine (6 mL) and was then concentrated under reduced pressure (23 °C, 40 mmHg), diluted with methanol (6 mL) and re-concentrated to give a crude solid. The solid was slurried in methanol (7.5 mL) and water (15 mL) was added dropwise with continuous stirring for 10 min. The slurry was stirred at room temperature for 1 h and allowed to crystallize according to Kuethe and Beutner, method [37]. It was filtered by suction and dried under vacuum/N_2 sweep for 8 h to afford *N,N*-diethyl substituted *p*-tolylsulfonamides **(8a-k).**

N,N-Diethyl-1-tosylpyrrolidine-2-carboxamide (8a)

Yield 0.89 g (92.3%), mp 114-116 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.76-7.73 (m, 2H, Ar-H), 7.33-7.31 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.28-7.26 (d, *J* = 8.0 Hz, 1H, Ar-H), 4.73-4.70 (m, 1H, CHa of CH_2-N), 4.26-4.24 (m, 1H, CHb of CH_2-N), 3.50-3.41 (m, 2H, $\underline{CH_2}$-CH_3), 3.34-3.29 (m, 2H, $\underline{CH_2}$-CH_3), 2.42-2.39 (d, *J* = 12.12 Hz, 3H, CH_3), 2.11-2.09 (m, 2H, CH_2 of pyrrolo), 1.94-1.71 (m, 3H, CH_2 & CH of pyrrolo), 1.27-1.23 (t, *J* = 7.2 Hz, 3H, $\underline{CH_3}$-CH_2), 1.09-1.06 (t, *J* = 7.12 Hz, 3H, $\underline{CH_3}$-CH_2). IR (KBr) cm^{-1}: 2974 (aromatic), 2866 (CH aliphatic), 1657 (C=O), 1609 (C=C aromatic), 1149, 1107 (SO_2 two bands), 673 (Ar-H). MS: in m/z [rel. %]: 225.0 [MH^+ - $CON(CH_2CH_3)_2$, 62%], 224.0 [M^+ - $CON(CH_2CH_3)_2$, 100%], 169.1 [89%], 155.0 [$PhCH_2SO_2^+$, 93%], 100.1 [$^+CON(CH_2CH_3)_2$, 17%], 91.0 [$PhCH_2^+$, 82%], 72.0 [45%], 65.0 [42%]. Anal. calcd. for $C_{16}H_{24}N_2O_3S$ (324.45): C, 59.23; H, 7.46; N, 8.63. Found: C, 57.97; H, 7.20; N, 7.92.

N,N-Diethyl-1-tosylpiperidine-2-carboxamide (8b)

Yield 0.94 g (94.1%), mp 127-129 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.58-7.56 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.21-7.19 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.86-4.85 (m, 1H, $\underline{CH}$-COOH), 3.73-3.70 (q, *J* = 7.2 Hz, 2H, $\underline{CH_2}$-CH_3), 3.31-3.26 (q, *J* = 7.08 Hz, 2H, $\underline{CH_2}$-CH_3), 3.15-3.14 (m, 1H, CHa of CH_2-N), 3.09-3.08 (m, 1H, CHb of CH_2-N), 2.36 (s, 3H, CH_3), 1.75-1.66 (m, 3H, CH & CH_2 of piperidine), 1.59-1.50 (m, 3H, CH & CH_2 of piperidine), 1.26-1.23 (t, *J* = 7.2 Hz, 3H, $\underline{CH_3}$-CH_2), 0.96-0.93 (t, *J* = 7.02 Hz, 3H, $\underline{CH_3}$-CH_2). Anal. calcd. for $C_{17}H_{26}N_2O_3S$ (338.47): C, 60.33; H, 7.74; N, 8.28. Found: C, 60.27; H, 7.60; N, 8.32.

N,N-Diethyl-2-(4-methylphenylsulfonamido) acetamide (8c)

Yield 0.69 g (82.7%), mp 109-111 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.75-7.73 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.29-7.27 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.80 (s-br, 1H, NH), 3.73-3.72 (d, *J* = 4.12 Hz, 2H, $\underline{CH_2}$-NH), 3.31-3.26 (q, *J* = 7.14 Hz, 2H, $\underline{CH_2}$-CH_3), 3.17-3.11 (q, *J* = 7.18 Hz, 2H, $\underline{CH_2}$-CH_3), 2.40 (s, 3H, CH_3-Ar), 1.12-1.08 (t, *J* = 7.18 Hz, 3H, $\underline{CH_3}$-CH_2), 1.02-0.99 (t, *J* = 7.14 Hz, 3H, $\underline{CH_3}$-CH_2). IR (KBr) cm^{-1}: 3034 (CH aromatic), 2946 (CH aliphatic), 1707 (C=O), 1601 (C=C aromatic), 1191, 1145 (SO_2 two bands), 694 (Ar-H). Anal. calcd. for $C_{13}H_{20}N_2O_3S$ (284.38): C, 54.91; H, 7.09; N, 9.85. Found: C, 55.03; H, 7.13; N, 9.92.

N,N-Diethyl-2-(4-methylphenylsulfonamido) propanamide (8d)

Yield 0.68 g (77.9%), mp 121-124 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.74-7.66 (m, 2H, Ar-H), 7.28-7.22 (m, 2H, Ar-H), 5.46-

5.44 (d, *J* = 8.48 Hz, 1H, NH-CH), 4.25-4.15 (m, 1H, NH-CH-CH_3), 3.99-3.92 (q, *J* = 7.12 Hz, 4H, 2 × CH_2-CH_3), 2.38 (s, 3H, CH_3-Ar), 1.39-1.37 (d, *J* = 7.16 Hz, 3H, CH_3-CH), 1.13-1.09 (t, *J* = 7.12 Hz, 6H, 2 × CH_3-CH_2). IR (KBr) cm^{-1}: 3279 (N-H), 3107 (CH aromatic), 1711 (C=O), 1620 (C=C aromatic), 1225, 1152 (SO_2 two bands), 677 (Ar-H). Anal. calcd. for $C_{14}H_{22}N_2O_3S$ (298.41): C, 56.35; H, 7.43; N, 9.39. Found: C, 56.42; H, 7.35; N, 9.44.

N,N-Diethyl-3-methyl-2-(4-methylphenylsulfonamido) butanamide (8g)

Yield 0.86 g (89.1%), mp 164-166 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.68-7.66 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.23-7.21 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.78-5.75 (d, *J* = 9.24 Hz, 1H, NH-CH), 3.81-3.78 (dd, J_1 = 4.22 Hz, J_2 = 9.24 Hz, 1H, NH-CH-CH), 3.15-3.02 (m, 4H, 2xCH_2-CH_3), 2.37 (s, 3H, CH_3-Ar), 1.83-1.78 (m, 1H, CH-CH$(CH_3)_2$), 1.03-1.01 (d, *J* = 6.8 Hz, 3H, CH_3-CH), 0.92-0.88 (t, *J* = 7.2 Hz, 3H, CH_3-CH_2), 0.84-0.82 (d, *J* = 6.0 Hz, 3H, CH_3-CH), 0.84-0.81 (t, *J* = 6.48 Hz, 3H, CH_3-CH_2). IR (KBr) cm^{-1}: 3260 (N-H), 2974 (CH aliphatic), 1668 (C=O), 1167, 1090 (SO_2 two bands), 689 (Ar-H). Anal. calcd. for $C_{16}H_{26}N_2O_3S$ (326.46): C, 58.87; H, 8.03; N, 8.58. Found: C, 58.79; H, 7.98; N, 8.59.

N,N-Diethyl-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (8j)

Yield 0.99 g (89.4%), mp 169-170 °C, ^{1}H-NMR ($CDCl_3$) δ: 7.67-7.65 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.24-7.19 (m, 5H, Ar-H), 7.13-7.09 (m, 2H, Ar-H), 5.88-5.86 (d, *J* = 9.48 Hz, 1H, NH-CH), 4.31-4.25 (m, 1H, CH), 3.21-3.15 (m, 1H, CHa of CH_2-Ph), 2.99-2.96 (m, 1H, CHb of CH_2-Ph), 2.94-2.90 (m, 2H, CH_2-CH_3), 2.80-2.74 (q, *J* = 7.2 Hz, 2H, CH_2-CH_3), 2.37 (s, 3H, CH_3-Ar), 0.88-0.84 (t, *J* = 7.14 Hz, 3H, CH_3-CH_2), 0.84-0.81 (t, *J* = 7.2 Hz, 3H, CH_3-CH_2). IR (KBr) cm^{-1}: 3306 (N-H), 2947 (CH aliphatic), 1710 (C=O), 1601 (C=C aromatic), 1213, 1171 (SO_2 two bands), 685 (Ar-H). Anal. calcd. for $C_{20}H_{26}N_2O_3S$ (374.51): C, 64.14; H, 7.00; N, 7.48. Found: C, 64.07; H, 6.91; N, 7.63.

Antibacterial Activity Assays

The antimicrobial properties of the sulfonamides were investigated in form of the general sensitivity testing[38] and minimum inhibitory concentration (MIC)[39] with respect to freshly cultured targeted organisms. The two organisms of interest in this present study are one gram positive (*Staphylococcus aureus* ATCC 6538) and one gram negative (*Escherichia coli* ATCC 25922) organisms which are associated with the gastrointestinal tract damage in man and animal.

Preparation of the inoculum

The standard strains of *S. aureus and E. coli* used were obtained from Test Center of Antimicrobial Materials, TIPC, Beijing. No clinically isolated organism was used based on in-availability of such as at the time of this study. The strains were propagated on nutrient agar plates and maintained on the plate at 4 °C. The isolates were sub-cultured in nutrient broth at 37 °C for 8 h prior to antibacterial testing.

Antibacterial sensitivity testing of the synthesized compounds

Agar well diffusion technique as described by Adeniyi and co-workers was used to determine the antibacterial activity of the synthesized compounds[38]. Sensitivity test agar plates were seeded with 0.1 mL of an overnight culture of each bacterial strain (equivalent to 10^7 – 10^8 CFU mL^{-1}). The seeded plates were allowed to set and a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.3 mL of each sulfonamide solution in appropriate solvent at a concentration of 1000 µg/mL (0.02g of sulfonamide dissolved in 20 mL DMSO). All the plates were incubated at 37 °C for 24 h. The assay was conducted at regular intervals of 24 h until marked decline in the potency of the sulfonamide solution to inhibit the growth of the test organisms was noticed. Zones of clearance round each well means inhibition and the diameter of such zones were measured. The procedure was repeated for the streptomycin (standard).

Determination of minimum inhibitory concentration (MIC)

Agar well dilution method as described by Russell and Furr was used to determine the minimum inhibitory concentration (MIC) of the sulfonamides and streptomycin[39]. Different dilutions of the sulfonamides were prepared first at ≤ 100 µg/mL to give final concentrations in the range of 100, 50, 25, 12.5 and 6.25 µg/mL. The different dilutions of sulfonamide derivatives that could not inhibit the microbial growth at ≤ 100 µg/mL were later prepared at ≤ 1000 µg/mL to give final concentrations in the range of 1000, 500, 250, 125, 62.5 µg/mL. Two milliliter (2 mL) of each dilution was mixed with 18 mL of Mueller Hinton agar (MHA, Difco, France) and poured into Petri-dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial strains and incubated overnight. The plates were then examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective sulfonamide. The procedure was repeated for streptomycin (standard). Selectivity index (S.I.) is the ratio of zone of inhibition of compound to that of the streptomycin.

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