Synthesis of *N*-aryl substituted *p*-toluenesulphonamides via nickel catalyzed amidation reaction and their antibacterial, antifungal and antioxidant activities evaluation

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Abstract: In present study nickel catalyzed synthesis of *N*-(aryl) substituted *p*-toluene sulphonamides is reported. The intermediate 4-methylbenzenesulphonamide (2) was obtained by the reaction between *p*-toluene sulphonyl chloride and ammonium hydroxide. Various readily available aryl halides (3a-e) Substituted *p*-toluene sulphonamides (4a-e) were obtained by coupling 4-methylbenezenesulphonamide (2) with via Buchwald-Hartwig cross-coupling reaction. Chemical structures of synthesized compounds were confirmed using Fourier Transform Infrared (FT-1R), proton and carbon-13 Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) and mass spectroscopy. The new compounds were screened for antibacterial and antifungal activities against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, salmonella typhi, candida albicans and Aspergillusniger* using agar diffusion technique. Some of the compounds showed higher activity when compared with the standards (ciprofloxacin and ketoconazole). The sulphonamides were further screened for antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl, Ferrous sulphate lipid per oxidation and Ferric reducing antioxidant power and the compound showed good antioxidant activity. Tests such as acute toxicity, liver function and kidney function was also carried out on the synthesized compounds.

Keyword: *p*-toluenesulphonamide, antioxidant, nickel catalysis, toxicity, antimicrobial.

INTRODUCTION

The history of drug research over a period of a century since Paul Ehrlich introduced the concept of chemotherapeutic agents is an amazing journey of accomplishments including the serendipitous success of antibiotics. The development of sulphonamides is a fascinating and informative area in medicinal chemistry (Bhat *et al.,* 2005). Its functional group has a long and rich history in organic chemistry and drug discovery (Eshaghi *et al.,* 2011, Hopper *et al.,* 2009). *p*-Toluenesulphonamides and benzenesulphonamides have been widely explored in synthetic chemistry (Juli*et al.,* 2011), and are known to represent a class of medicinally important compounds which are extensively used as antibacterial agents (Cohan *et al.,* 2010). Sulphonamides are widely used in medicinal chemistry because of their low cost, low toxicity and excellent biological activities. Many infectious diseases caused by bacteria are cured by widely use of sulphonamides. In addition to their use as antibacterial agents, sulphonamides are widely used as anticancer (Fukuoka *et al.,* 2001), carbonic anhydrase inhibitors (Weber *et al.,* 2004), antimalarial (Padmanilayan *et al.,* 2006), antitumor (Bouchain*et al.,* 2003), antihypertensive (Banerjee *et al.,* 2005), anti-inflammatory (Inabaj *et al.,* 2000), and antiprotozoal agents (Chibale *et al.,* 2001). They have been used against most gram-positive and many gram-negative organisms, fungi and certain protozoa (Perlovich *et al.,* 2008). Nassir *et al.,* 2013 reported a series of bis-benzimidazole and bis-imidazole sulphonamides that were found to be active against gram-positive and gram-negative bacteria by treating imidazole or benzimidazole with tris (4-substituted benzensulfonate)-diethanolamine under basic condition. Zareef *et al.,* 2006 reported the synthesis of novel primary benzene sulphonamide bearing 2,5-disubstituted-1,3,4-oxadiazole moiety which has a good anti HIV activity. Fors *et al.,* 2009 successfully used a new biarylphosphine ligand (*t*-Bu Brettphos) for palladium catalyzed cross coupling reactions of 1-chloro-2-methylbenzene and acetamide to produce *N*-phenylacetamide. They reported that this system shows the highest turnover to date for these reactions, especially for aryl chloride substrates bearing an ortho substituent. Furthermore, sulphonamides are also highly relevant both in the animal world and plant life cycle. In fact, the breakdown of cyclic guanosine monophosphate is retarded by sildenafil, a substituted guanine analog, which indeed keeps cut flowers fresh for another week and also strengthens plant stem to stand straight even in the midst of storm and wind (Bergmann, 2010). A preserving effect on fruit and vegetables was also found, making sildenafil a promising agent (Bergmann, 2010). Today, it is marketed under the trade name Viagra which is a potent drug used in the treatment of erectile dysfunction in men (Blonde, 2006). Palladium catalyzed reaction has been one of the most widely used protocol in the formation of C-N bond in organic synthesis. On the other hand, nickel catalyzed reaction have received less attention. At present,

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there is little report of nickel catalyzed tandem reactions for the formation of C-N moiety in compounds. It is the interest in this direction that prompted the present synthesis of *p*-toluenesulphonamide derivatives via the nickel catalyzed reaction employing the Buchwald-Hartwig protocol.

MATERIALS AND METHODS

General consideration

All the chemicals used were obtained from Sigma-Aldrich and BDH laboratories and were used without further purification. Melting point was determined using Fischer John's melting point apparatus and was uncorrected. IR spectra were recorded on 8400s Fourier Transform Infrared (FT-IR) spectrometer using potassium bromide disks at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, and are reported in wave number (cm^{-1}). Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) were determined using Varian 400MHz at Strathclyde University, Scotland. Chemical shifts are reported in (δ) scale. The mass spectroscopy was determined at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State.

Synthesis of 4-methylbenzenesulphonamide (2)

To *p*-toluenesulphonamide (3.81g, 20mmol), placed in a 100mL Erlenmeyer flask, ammonium hydroxide (1.4g, 40mmol) was added. The mixture was stirred well with a stirring rod for 5 min. After which the mixture warms up slightly and thicken to a paste that was difficult to stir, 20 mL of distilled water was added and stirring continued for 3min. The mixture was warmed on a water bath to 60^{o}C for 2 min and chilled in ice-water. The solid was collected in a Buchner funnel (suction filtration), air dried and recrystallized from aqueous ethanol to give 4-methylbenzenesulphonamide (2) as a crystalline low melting solid. Yield (3.27g, 96%), m.p. 58-60^{o}C. IR (KBr) $\nu_{max}cm^{-1}$: 3446, 3337 (N-H stretch of NH_2), 3072 (C-H aromatic), 2992, 2942 (C-H aliphatic), 1585 (C=C aromatic), 1373 (S=O), 1172(SO_2NH), 813 (C-H deformation), 650 (*p*-substitution in benzene). ^{1}H-NMR (DMSO-d_6)δ: 14.51 (s, 2H, NH_2), 7.51 (d, J=7.86 Hz, 2H, Ar-H), 7.12 (d, J=7.86 Hz, 2H, Ar-H), 2.24 (s, 3H, CH_3-Ar). ^{13}C–NMR (DMSO-d_6)δ: 144, 139, 130, 129, 127, 126, 21. M+ ($C_7H_9NO_2S$): 171; logP: 0.79±0.21.

Synthesis of bis (triphenylphosphine) nickel(II) chloride

This compound was prepared according to the procedure developed by Vananzi1958. Triphenylphosphine (5.25g, 20mmol) was placed in a 250mL beaker containing 75 mL glacial acetic acid; the mixture was heated until the triphenylphosphine dissolved completely. Nickel (II) chloride hexahydrate (2.38g, 10 mmol) dissolved in 2 mL of distilled water was added. The mixture was set aside for 24 h and filtered using suction. The dark green crystals collected in the Buckner funnel was dried in a desiccator.

General procedure for the synthesis of derivatives (4a-e)

Bis (triphenylphosphine) nickel(II) chloride (0.3g,) and triphenylphosphine (0.9g, 3mmol) were placed in 100 mL two necked flask equipped with a magnetic stir bar. The solvent (*t*-butanol (4mL) and water (2mL)) was added via 10mL syringe and the mixture stirred for 5 min at room temperature under nitrogen atmosphere. Afterwards, it was heated at 80^{o}C for 90 min. Thereafter, 4-methyl benzenesulphonamide (2) (1.71g, 10mmol), potassium carbonate (1.38g, 10mmol) and substituted aryl halides (3a-e) were added to the mixture with the solvent *t*-butanol and water in the ratio of 2:1 under inert atmosphere. The mixture was refluxed with stirring for 1 h at a temperature of 110^{o}C. The mixture was then cooled at room temperature, diluted with ethyl acetate, washed with water to afford aryl substituted *p*-toluenesulphonamides in good yields. The products were purified from aqueous methanol.

N-(4-Aminophenyl)-4-methylbenzenesulphonamide (4a)

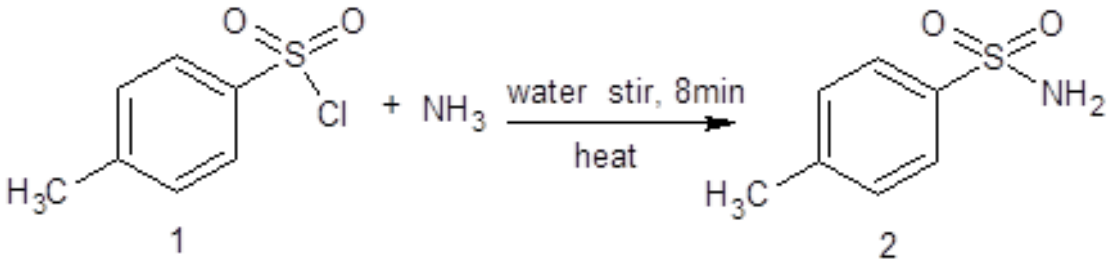
Using 2, bis (triphenylphosphine) nickel(II) chloride, 4-bromoaniline (3a) andtriphenylphosphine as starting materials, the title compound 4a was obtained as a thick black oil; yield 2.0 g (78 %). IR (KBr) $\nu_{max}cm^{-1}$: 3441 (N-H stretch), 3361, 3222 (N-H Stretch of NH_2), 3055 (C-H aromatic), 2910 (C-H aliphatic), 1613, 1472 (C=C of aromatic), 1308 (S=O), 1159 (SO_2NH), 1019 (C-N stretching), 923, 822 (C–H deformation), 710 (substitution in benzene). ^{1}H NMR (DMSO-d_6)δ: 7.63 (m, 3H, ArH), 7.33 (d, J=8.19 Hz, 2H, ArH), 7.12 (m, 4H, ArH), 6.52 (m, 4H, ArH), 5.27 (s, IH, NH), 2.33 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6)δ: 154.24, 142.09, 142.01, 132.98, 132.21, 130.11, 129.09, 129.01, 127.20, 126.94, 120.34, 120.04, 21.02. M+ ($C_{13}H_{14}N_2O_2S$): 262; logP, 1.36±0.32

N-(4-Formylphenyl)-4-methylbenzenesulphonamide (4b)

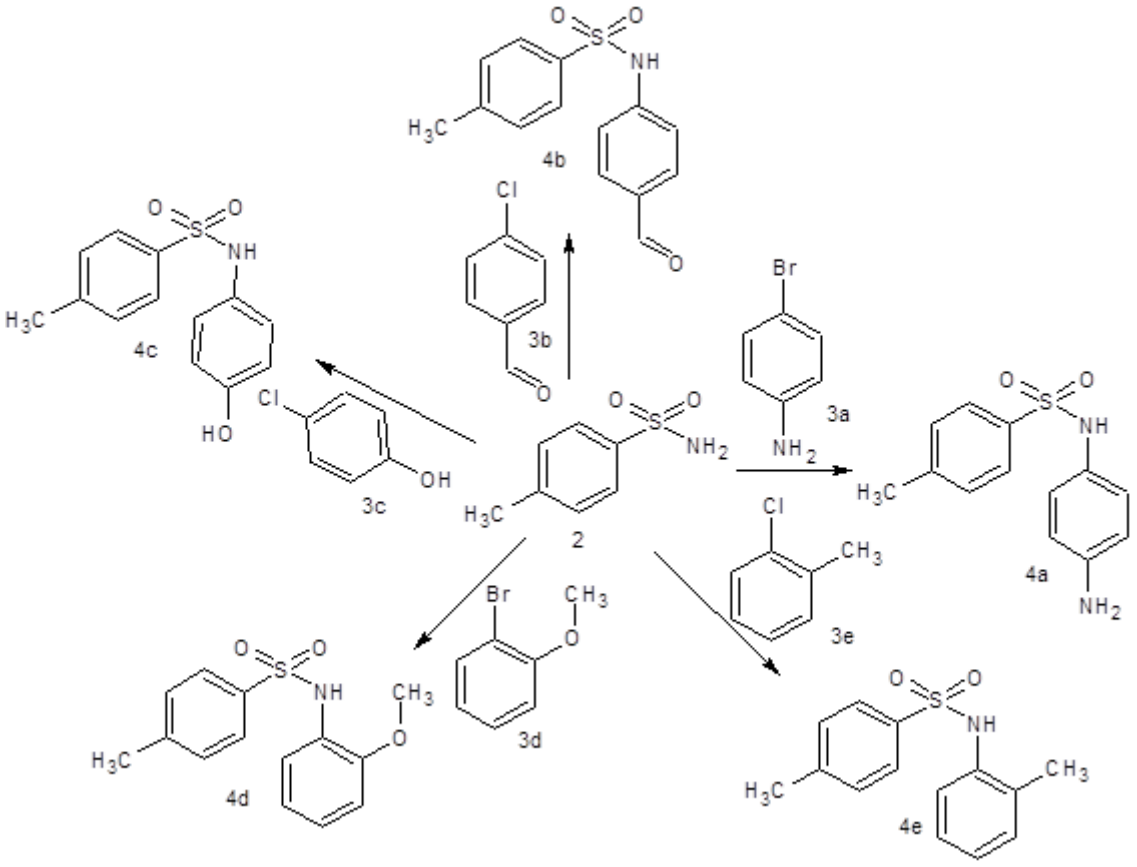
Using 2, bis (triphenylphosphine) nickel(II) chloride, 4-chlorobenzaldehyde (3b) andtriphenylphosphine as starting materials, the title compound 4b was obtained as a light-yellow oil; yield 1.90 g (69 %). IR (KBr) $\nu_{max}cm^{-1}$: 3393(N–H stretching), 3060 (C-H aromatic), 2846 (C–H aliphatic), 1696 (C=O), 1589, 1440 (C=C of aromatic), 1307 (S=O), 1172 (SO_2NH), 1011 (C-N), 828 (C–H deformation), 716 (substitution in benzene ring). ^{1}HNMR (DMSO–d_6)δ: 10 (s, 1H, NH), 7.92 (dd, J_1 = 1.93 Hz, J_2 = 8.60 Hz, 2H, ArH), 7.59 (m, 4H, Ar-H), 3.80 (d, J = 32.14Hz, 1H, CHO), 2.32 (s, 3H, CH_3-Ar). ^{13}C NMR (DMSO-d_6)δ: 167.04, 152.08, 150.45, 142.32, 140.87, 136.45, 135.90, 135.21, 134.56, 134.05, 128.75, 128.10, 127.90, 20.92. M+ ($C_{14}H_{13}NO_3S$): 275; logP, 2.96±0.37

N-(4-Hydroxylphenyl)-4-methylbenzenesulphonamide (4c)

Using 2, bis (triphenylphosphine) nickel(II) chloride, 4-chlorophenol (3c) andtriphenylphosphine as starting materials, the title compound 4c was obtained as a



Scheme 1: Synthesis of 4-methyl benzenesulphonamide



Scheme 2: Synthesis of various *N*-aryl substituted 4-methyl benzenesulphonamides (4a-e).

colourless oil; yield 1.97g (74.9%). IR (KBr) ν_{max}cm^{-1}: 3084(C-H aromatic), 2684, 2796 (C-H aliphatic), 1594, 1467 (C=C aromatic), 1249 (S=O), 1166 (SO_2NH), 1009 (C-O), 838 (C-H deformation), 724 (Substitution in benzene). ^{1}HNMR (DMSO-d_6)δ: 9.75 (s, 1H, NH), 7.56 (m, 4H, ArH), 6.79 (d, J=8.70Hz, 2H, ArH), 2.31 (s, 3H, CH_3-Ar). ^{13}C NMR (DMSO-d_6)δ: 145.25, 137.09, 137.01, 135.76, 134.92, 133.76, 133.24, 132.09, 132.01, 123.71, 123.02, 121.07, 23.06. M+ ($C_{13}H_{13}NO_3S$): 263; logP, 2.30± 0.31

N-(2-Methoxyphenyl)-4-methylbenzenesulphonamide (4d)

Using 2, bis (triphenylphosphine) nickel(II) chloride, 2-bromoanisole (3d) andtriphenylphosphine as starting materials, the title compound 4d was obtained as a yellow oil; yield 1.99 g (72 %). IR (KBr) ν_{max}cm^{-1}: 3403(N–H stretch), 3056 (C–H of aromatic), 2945, 2852 (C–H aliphatic), 1687, 1588, 1462 (C=C aromatic), 1274 (S=O), 1033 (C-O), 1155 (SO_2NH), 726 (substitution in benzene). ^{1}HNMR (DMSO–d_6)δ: 7.62 (m, 4H, Ar-H), 7.52 (td, J_1=2.38 Hz, J_2=7.09 Hz, 4H, ArH), 4.38 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3-Ar). ^{13}C–NMR (DMSO–d_6)δ: 145, 142, 140, 138, 136, 133, 132, 131, 130, 129, 128, 126, 21 and 20. M+ ($C_{14}H_{15}NO_3S$): 277; logP, 3.05±0.32

4-Methyl-N-(2-methylphenyl) benzenesulphonamide (4e)

Using 2, bis (triphenylphosphine) nickel(II) chloride, 2-chlorotoluene (3e) and triphenylphosphine as starting materials, the title compound 4e was obtained as a waxy low melting solid; yield 1.56 g (59%). IR (KBr) ν_{max}cm^{-1}: 3401 (N-H stretch) 3057 (C-H aromatic), 1668, 1452 (C=C of aromatic), 1319 (S=O), 1171 (SO_2NH), 1016 (C-N), 817 (C-H deformation), 705 (Substitution in benzene).

^{1}HNMR (DMSO–d_6)δ: 7.57 (m, 4H, ArH), 4.21 (s, 3H, CH_3-Ar), 2.30 (s, 3H, CH_3-Ar). ^{13}CNMR (DMSO–d_6)δ: 145, 142, 140, 138, 136, 133, 132, 130, 129, 128, 127, 126, 22 and 21. M+ ($C_{14}H_{15}NO_2S$): 261; logP, 3.50± 0.30

Antimicrobial activity

Agar cup diffusion technique as described by Ugwu *et al* 2014 was used to determine the antimicrobial activity of the synthesized compounds. Sensitivity test agar plates were seeded with 0.1mL of overnight culture of microorganisms. The seeded plates were allowed to set after which cups were made in each sector previously drawn on the backside of the bottom plate using marker. Using a sterile pipette, each cup was filled with six drops (2mL) of their corresponding synthesized compound (100 mg/mL) using DMSO as the solubilising agent. All the plates were incubated at 37^{o}C for 24 h for bacteria and 48 h for fungi. Zones of clearance round each cup means inhibition and the diameter of such zones were measured. The graph of IZD^2 against the log of concentration was plotted for each plate containing specific compound and a microorganism. The anti-log of the intercept on the x-axis gave the MIC. The procedure was repeated for ciprofloxacin (standard antibacterial agent) and ketoconazole (standard antifungal agent).

Antioxidant activity studies

The synthesized compounds were further evaluated for the antioxidant potential by various *in vitro* assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferrous sulphate lipid per oxidation assay and Ferric ion (Fe^{3+}) reducing antioxidant power assay (FRAP) using ascorbic acid as reference drug.

DPPH radical scavenging activity

The method of Liyana-Pathiranan and Shahidi 2005 was used for the determination of scavenging activity of DPPH free radical. A solution of 0.135mL DPPH in methanol was prepared and 1.0mL of this solution was mixed with 1.0mL of sample prepared in methanol containing 0.025-0.5mg of the sulphonamides and standard drug (ascorbic acid) separately. The reaction mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability of the sulphonamide to scavenge DPPH was calculated by the equation:

$$\text{DPPH radical scavenging activity} = \frac{(Abs_{ccontrol}) - (Abs_{sample})\ x100}{Abs_{control}}$$

Where $Abs_{control}$= the absorbance of DPPH radical + methanol

Abs_{sample}= the absorbance of DPPH radical +sulphonamide compounds or standard.

Ferrous sulphate induced lipid peroxidation scavenging

The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS) using the standard methods (Okhawa *et al.*, 1979) with minor modifications (Tripathi and Sharma, 1998). Briefly, different concentrations of sulphonamide extracts (25-400, µg/mL) were added to the 10% liver and kidney homogenate. Lipid peroxidation was initiated by adding 100 µL of 15mM $FeSO_4$ solution to 3mL of liver homogenate (final concentration was 0.5mM). After 30 min, 100µL of this reaction mixture was taken in a tube containing 1.5mL of 0.67% TBA in 50% acetic acid. The mixture was heated in a water bath at 85^{o}C for 30 min and in a boiling water bath to complete the reaction. The intensity of pink coloured complex formed was measured at 535nm in a spectrophotometer. The percentage inhibition of lipid per oxidation was calculated, as per the following formula

$$\text{Inhibition (\%)} = \frac{(Control - test)\ x\,100}{Control}$$

FRAP Assay

The sulphonamide compounds were screened for ferric reducing antioxidant power. The appropriate amounts of compounds 2 and 4a-e were mixed with phosphate buffer (2.5mL, 0.2M, pH =6.6) and potassium ferric cyanide (2.5mL, 1%). The mixture was incubated at 50^{o}C for 20 min. Later, the reaction mixture was acidified with trichloroacetic acid (2.5mL, 10%). After $FeCl_3$ (0.5mL, 0.1%) was added to this solution, the absorbance was measured at 700nm. The increased absorbance of the reaction mixture indicates an increased reducing power.

Acute toxicity studies (LD_{50})

Experimental Animals

Healthy adult abino rats weighing between 150 and 260 g were obtained from the Animal House of the University of Nigeria, Nsukka. The animals were housed under standard environmental conditions, and feed and water were provided. Proper handling and using of the animals were in accordance with the guidelines and committee on animal use of University of Nigeria, Nsukka. The albino rats were housed in cages and randomly selected ones were marked on the tail for individual identification. The animals were allowed to acclimatize to laboratory conditions for a week before starting the experiment

Experimental design for acute toxicity study (LD_{50})

The method of Lorke 1983 was used to investigate the oral dose of sulphonamide derivatives that produced immediate or acute toxicity in rats. The animals were divided into three groups of three rats each and treated with respective doses of (200, 400, and 600) mg/kg body weight of the aqueous extract of sulphonamide by oral route. In the second investigation (after 24h), three groups were also used with three animals per groups. The following doses were used; 1000, 2000 and 3000mg/kg respectively. Animals were observed on hourly basis for the first day and afterwards, daily for six more days. Mortality or any visible sign of injury were recorded.

RESULTS

Table 1: Minimum Inhibitory Concentration (mg/mL)

Compounds	*S. aureus*	*S. typhi*	*P. aeruginosa*	*E. coli*	*K. pheumoniae*	*C. albicans*	*A. niger*	*logP*
2	5.75	6.02	10.00	15.84	20.08	+	+	0.79±0.21
4a	3.01	+	10.00	+	+	13.80	5.75	1.36±0.32
4b	+	+	+	5.01	+	+	+	2.96±0.37
4c	15.84	15.80	17.37	+	13.80	+	+	2.30±0.31
4d	+	+	14.45	+	+	+	+	3.05±0.32
4e	19.95	+	10.00	+	14.45	+	+	3.50±0.30
CIP	13.80	15.84	12.58	7.94	13.80	+	+	
KET	+	+	+	+	+	14.45	7.94	

+ implies no activity or resistance, KET implies ketoconazole whereas CIP means ciprofloxacin.

Table 2: IC_{50} values of DPPH and $FeSO_4$ radical scavenging

Test compounds	Scavenging activity (IC_{50})	
	DPPH	$FeSO_4$
2	-0.25	8.62
4a	1.58	35.44
4b	2.50	8.17
4c	6.33	6..83
4d	3.44	5.57
4e	10.94	32.65
Abscorbic acid	0.02	8.50

Table 3: FRAP reducing ability Fe^{3+} -Fe^{2+} Reducing ability

Test compounds	1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL
2	0.052	0.103	0.033	0.005	0.094
4	0.068	0.179	0.161	0.104	0.106
4b	-0.093	0.016	0.068	0.063	0.114
4c	0.153	0.161	0.167	0.172	0.117
4d	0.179	0.160	0.060	0.113	0.048
4e	0.047	0.103	0.117	0.079	0.057
Ascorbic acid	0.205	0.253	0.300	0.384	0.584

Table 4: LD_{50} values of compounds 2 and 4a-e

Tested Compounds	LD_{50} values, (mg/kg)
2	2685.34
4a	386.38
4b	1492.56
4c	650.84
4d	2527.54
4e	1926.01

Table 5: Hodge and Sterner Toxicity Scale (1980)

Toxicity rating	Commonly used term	LD_{50} (rat, oral)
1	Extremely Toxic	Less than 1mg/kg
2	Highly Toxic	1-50 mg/kg
3	Moderately Toxic	50-500mg/kg
4	Slightly Toxic	500-5000mg/kg
5	Practically Non-toxic	5000-15000mg/kg

Table 6: Effect of the new sulphonamides on serum AST, ALT and ALP levels of rats (dose = 200 mg/kg).

	2	4a	4b	4c	4d	4e	control
AST	33± 1.00	70 ±1.67	57±1.00	41±2.00	35±0.83	42±1.83	40±1.65
ALT	12.5±0.99	21.9±0.10	20.2±0.40	18.4±0.80	14.6±0.60	18.9±0.42	20.0±0.75
ALP	0.56±0.014	1.54±0.012	0.79±0.014	0.80±0.016	0.59±0.009	0.72±0.024	0.82±0.014

Table 7: Effects of the new sulphonamides on serium urea, creatinine and uric acid levels of rats (dose = 200mg/kg)

	2	4a	4b	4c	4d	4e	control
Urea	18.60±0.008	19.80±0.012	17.70±0.04	17.50±0.008	16.90±0.002	18.40±0.002	19.05±0.008
Creatinine	1.17±0.39	1.45±0.33	1.58±0.39	1.64±0.33	1.36±0.39	1.43±0.11	1.50±0.05
Uric acid	5.40±0.02	5.70±0.004	5.90±0.001	6.55±0.02	6.20±0.003	6.55±0.03	6.40±0.005

All data were expressed as mean ± S. E. (standard error). Unit = IU/L

Liver function tests (LFTs)

The liver function tests carried out with the blood of the rats fed with the sulphonamide derivatives were aspartate aminotransperase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Standard laboratory procedure according to Reitman and Franke 1957 was used for the determination of AST, ALT and ALP.

Renal or kidney function test

Kidney function tests carried out with the blood of the rats fed with the sulphonamide derivatives were serum urea, creatinine and uric acid. The method reported by Kaplan and Tengv1982 was used in the determination of urea and creatinine while that reported by Ochie and Kolhattar2000, was used for the determination of uric acid.

DISCUSSION

We described here the synthesis of various biologically active novel *N*-aryl substituted 4-methylbenzenesulphonamides via Buchwald-Hartwig protocol using 4-methylbenzenesulphonyl chloride as the starting material.

On stirring 4-methylbenzenesulphonyl chloride (1) with ammonium hydroxide in water for 8 min, and on heating the mixture for 5 min, 4-methylbenzene-sulphonamide (2) was obtained as a crystalline low melting solid (scheme 1).

The water promoted activation of bis (triphenylphosphine) nicke (II) chloride is shown below $NiCl_2$ $(PPh_3)_2$, + $2PPh_3$ +H_2O⟶$(Ph_3P)_2$ $Ni^{(O)}$ + 0 = PPh_3 + 2HCl As shown in (scheme 2), a mixture of bis (triphenylphosphine) nicke(II) chloride and triphenylphosphine in a solvent of *t*-butanol and water was pre-activated by continuous stirring for 5 min under nitrogen atmosphere, followed by heating at a temperature of 80^{o}C for 90 min. 4-Methyl benzenesulphonamide (2), appropriate aryl halides (3a-e) and potassium carbonate were added with a further addition of *t*-butanol-water. On refluxing with stirring for 1h at 110^{o}C, the corresponding *N*-aryl substituted 4-methyl benzenesulphonamides (4a-e) were obtained and purified from aqueous methanol. The structures of the sulphonamides were determined using FTIR, ^{1}HNMR and ^{13}CNMR. Compounds 2 and 4a-e were screened for biological activities such as antibacterial, antifungal and antioxidant activities. Tests like acute toxicity, liver and kidney function tests were also carried out to ascertain the biosafety of the compounds.

Antimicrobial activity

4-Methyl benzenesulphonamide (2) and *N*-aryl substituted 4-methyl benzenesulphonamides (4a-e) alongside with ciprofloxacin and ketoconazole were screened in vitro for their antibacterial and antifungal activities on the test organisms *(Klebsiella pneumonia, Pseudomonas aeurginosa, Escherichia coli, Staphylococcusaureus, Salmonella typhi, Candida albican* and *Aspergillusniger)* using the agar diffusion techniques.

The antibacterial and antifungal activities of the synthesized compounds were compared with standard drugs (ciprofloxacin and ketoconazole) and the results of the investigations are presented in table 1. The MIC of most of the sulphonamides when compared with ciprofloxacin was found to be more active than the standard even though some of the sulphonamides were inactive against some organisms.

The antifungal activities of the sulphonamides were determined using *C. albicans* and *A. niger*, and only compound 4a was found to be active against these organisms. Compound 4a displayed more activity than ketoconazole (standard).

Antioxidant activities

DPPH free-radical scavenging activity

The DPPH radical scavenging activity assay is a simple method for measuring the compounds ability to trap free radicals. The scavenging effects of compounds (2, 4a-e) and that of the control (ascorbic acid) are presented in table 2. Compound 2 showed higher activity than the standard with $1C_{50}$ value of (-0.25 mg/mL), compounds 4a, 4b and 4d containing amino group, formyl group and methoxy group on the phenyl ring respectively displayed

high promising activity with $1C_{50}$ values of (1.58, 2.50 and 3.44) mg/mL and compounds 4c and 4eshowed the least activity when compared with the standard (ascorbic acid).

$FeSO_4$ induced lipid perioxidation

Lipid peroxidation (LPO) has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bio-enzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. Initiation of lipid peroxidation by ferrous sulphate takes place through ferrylperfearyl complex (Zbinden and Roversi, 1981). The lower the IC_{50} value the better the radical scavenging activity. The $FeSO_4$ radical scavenging capacity of compounds 2 and 4a-e are shown in table 2. From the table, it is evident that compounds 4c and 4d had better radical scavenging activity against ferrous sulphate lipid peroxidation than reference drug whereas compounds 2 and 4b had comparable activity with ascorbic acid.

Ferric reducing antioxidant power (FRAP)

Ferric reducing power was determined using the Fe(III) to Fe(II)reduction assay. Since the antioxidant activity of a substance is usually correlated directly to its reducing capacity, the FRAP assay provides a reliable method to study the antioxidant activity of various compounds. Ferric reducing ability of compounds (2, 4a-e) at different concentration were studied (table 3). At concentration 1mg/mL, compound 4d showed the best reducing power with absorbance value of 0.179 when compared with standard, at 2mg/mL, compound 4a showed the best reducing power with the absorbance value of 0.179, at concentrations 3mg/mL, 4mg/mL and 5mg/mL, compound 4c showed the best reducing power with absorbance values of 0.167, 0.172 and 0.117 respectively. High absorbance indicates high reducing power.

Acute toxicity investigation (LD_{50})

Median Lethal dose (LD_{50}) is the dose given all at once, which cause the death of half the number of test animals. LD_{50} is one way to measure the short-term toxic potential of a product (Gutteridge, 1985). The acute toxicity of compounds 2 and 4a-e were determined using LD_{50} calculation and the results are given in table 4.

According to Hodge and Sterner toxicity scale, the LD_{50} values of the sulphonamide derivatives are in the slightly toxic category (table 5) except for compound 4a that falls in the moderately toxic category. In general, the LD_{50} values of the newly synthesized compounds suggest that they are relatively safe toxicologically.

Liver function tests evaluation

Liver function tests are a group of blood tests that detect inflammation and damage to the liver. They can check how well the liver is working. The liver function tests studied in this research are AST, ALT and ALP. It is observed from table 6 that the administration of 200 mg/kg of the sulphonamide to the tested rats did not show significant increment in the serum levels of AST, ALT and ALP when compared with the control except for compound 4a which showed drastic increase in the AST level of the rat.

Kidney function test evaluation

Kidney function tests are common lab tests used to evaluate how well the kidneys are working. In this research work, the kidney function tests used are blood urea nitrogen (BUN), creatinine, and uric acid. In the rats fed with 200 mg/kg of sulphonamide derivatives, there were no significant changes for the serum levels of urea, creatinine and uric acid when compared with the control.

CONCLUSION

The synthesis of 4-methylbenzensulphonamide (2) and the nickel catalyzed transformation to *N*-aryl substituted *p*-toluene sulphonamides (4a-e) *via* Buchwald-Hartwig tandem amidation protocol has been achieved successfully. The structures assigned to these compounds have been supported by spectral analysis. The synthesized compounds were found to have good antimicrobial activities with only compound 4a having good antifungal activity against *C. albicans and A. niger*. The new compounds were screened for antioxidant activities and showed improvement when compared with ascorbic acid. The toxicity test showed that the compounds were slightly toxic and therefore could be a potential anticancer agent.

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