IJPSR (2018), Volume 9, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 April 2018; received in revised form, 13 July 2018; accepted, 13 November 2018; published 01 December 2018

SYNTHESIS AND BIOLOGICAL SCREENING OF PYRIMIDINE LINKED BENZENE SULFONAMIDE DERIVATIVES

Jyothi Boggavarapu [1] and Madhavi Nannapaneni [*2]

Department of Chemistry [1], Swarna Bharathi Institute of Science and Technology, Khammam - 507002, Telangana, India.
PG Department of Chemistry [2], J. K. C. College, Guntur - 522006, Andhra Pradesh, India.

Keywords:

Pyrimidine, Sulfonamide, Microwave irradiation, Antibacterial, Antifungal activity

Correspondence to Author:
Dr. Madhavi Nannapaneni

HOD.
PG Department of Chemistry,
J. K. C. College, Guntur - 522006,
Andhra Pradesh, India.

E-mail: madhavijkcchempg@gmail.com

ABSTRACT: Background: It has been developed a new combination of Palladium catalyzed Buchwald-Hartwig type reaction for the synthesis of *N-tert*-Butyl- 3- {[5- methyl- 2- (arylamino)pyrimidin- 4- yl]amino}benzene-sulfonamides 5 by the treatment of *N-tert*-butyl-3-[(2-chloro-5-methyl pyrimidin-4-yl)-amino]benzene sulfonamide 4 with various aromatic amines in the presence of Cs_2CO_3 and in DMF under microwave conditions. **Method:** All the eight compounds 5a-h were screened *in-vitro* for their antibacterial Gram-positive bacteria namely, *Bacillus subtilis, Bacillus sphaericus and Staphylococcus aureus* and three Gram-negative bacteria *Pseudomonas aeruginosa, Klebsiella aerogenes, Chromobacterium violaceum.* All the synthesized compounds were tested for their antifungal activity against five test organisms, *Aspergillus niger, Chrysosporium tropicum, Rhizopus oryzae, Fusarium moniliforme and Curvularia lunata.* **Results:** Among the title compounds 5d and 5e exhibited potent activity towards both gram positive and gram negative bacteria. Compounds 5e and 5f showed good antifungal activity. **Conclusion:** A new efficient catalyst/ligand combination was developed for the synthesis of title compounds 5a-5h under microwave conditions. The microwave procedure is slightly superior to the conventional method in terms of reduced time period and better yields.

INTRODUCTION: Nitrogen possessing hetero-cyclic compounds have received prominent attention due to their extensive pharmacological activity. Pyrimidine systems have been received particular attention and widely recognized as biologically useful systems, since they are important structural components of naturally occurring nucleic acids, variolin related alkaloids, meridianins also possess this frame work and these are cyclin-dependent kinase (CDKs) inhibitors [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(12).5534-43

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(12).5534-43

It is also an important constituent in vitamins like thiamine (vitamin B_1), riboflavin and folic acid. Synthetic compounds which contain pyrimidine skeleton such as imatinib, zidovudine and trimethoprim are important drugs used as anticancer, antiviral and antibiotic **Chart 1** agents. They play a pivotal role in the antiquity of heterocyclic chemistry. They are substantially used as synthons in organic chemistry field.

The pyrimidine derivatives are also have been found to exhibit a wide range of pharmacological activities, such as anti-bacterial [2], anti-inflammatory [3], antiproliferative [4], anti-cancer [5], leishmanicidal [6], antifungal [7], anti-convulsant [8], cycotoxic [9], anti-tubercular [10], anti-oxidant [11] and diuretic [12] activities.

These compounds are also used as hypnotic drugs for the nervous system [13], calcium-sensing receptor antagonists [14] and antagonists of the human A2A adenosine receptor [15].

Sulfonamides (sulfa drugs) are synthetic antimicrobial agents, derived from sulphanilamide that inhibits the growth of bacteria due to presence of NH and SO_2 group of sulfonamide. It eliminates bacteria that cause infections by stopping the production of folate inside the bacterial cell. The sulfonamide derivatives have been reported with diverse structural features and versatile biological properties such as antiplasmodial [16], carbonic anhydrases I, II, IV and IX inhibitors [17], antioxidant, anticholinesterase [18], antitumor, anti-proliferative [19] activity.

The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. It involves efforts to combine separate pharmacophoric groups of similar activity into one compound, thus making structural changes in the biological activity. So, the discovery of novel and potent antimicrobial agents is the best way to overcome microbial resistance and develop effective therapies.

Palladium catalyzed Buchwald-Hartwig organic transformations are the traditional methods to assemble these compounds for the formation of carbon-carbon and carbon-heteroatom bonds. Facile aromatic C-N bonds are synthesized by cross coupling of aryl halides with amines. These reactions involved, heating the substrate at high temperature for a longer period of time in which many functional groups were affected, and therefore their usage was greatly limited.

Microwave irradiation [20] is an energy source of which the popularity and synthetic utility in organic chemistry has increased considerably in recent years for both lead identification and optimization processes of new organic small molecules [21]. The rapid heating induced by such radiation avoids the harsh conditions and reagent decomposition of classical methods, reduces reaction time [22], leading to the formation of products under mild reaction conditions and normally with increased yields [23].

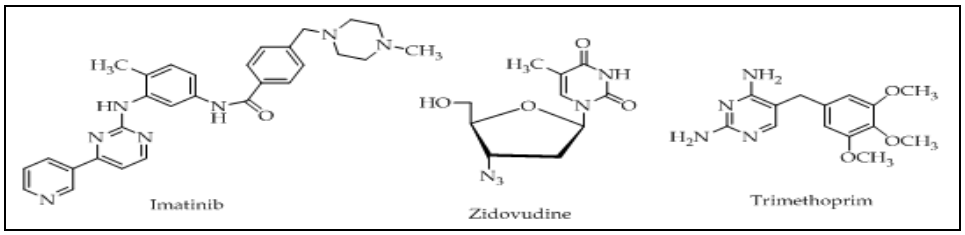


CHART 1: CHEMICAL STRUCTURES OF IMATINIB, ZIDOVUDINE AND TRIMETHOPRIM

Substituted pyrimidines are already well established as key cores in medicinal chemistry, along with that the sulfonamides have lot of biological significance and in connection with present search on the design and synthesis of substituted pyrimidines linked to sulfonamide in a single molecular frame work. It was envisaged that these two active pharmacophores, if linked together, would generate novel molecular templates which are likely to exhibit interesting biological properties.

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents has prompted studies on the development of new potential antimicrobial compounds. An attempt was made to synthesize and evaluate biological activities of novel N-tert-butyl-3-{[5-methyl-2-(arylamino)- pyrimidin- 4- yl] amino} benzene sulfonamides 5.

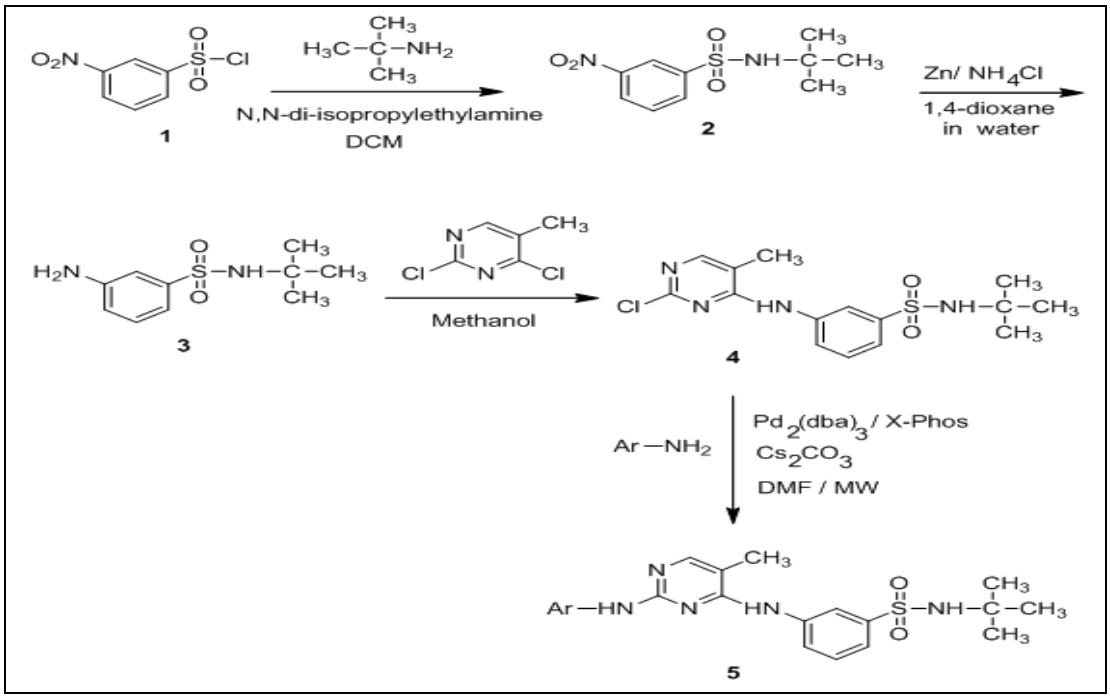
MATERIALS AND METHODS: Melting points were determined using a Cintex melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed by using Merck silica gel 60 F254 precoated plates (0.25 mm) and column chromatography was performed by using Silica gel (particle size 100-200 mesh). IR spectra (KBr) were recorded on a Perkin-Elmer BX series FTIR spectrometer. ^{1}H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker AMX 100 MHz spectrometer. Chemical shift values were given in ppm (δ) with TMS as an internal standard. Mass spectra were determined on Agilent LC-1100 (LC-MS) series instrument.

Elemental analyses were performed on a Carlo Erba 106 and Perkin Elmer model 240 analyzers. Anhydrous DMF (company name) was purchased and was used without further purification. Tris (dibenzylidene-acetone) dipalladium (0), X-Phos and Cs_2CO_3 (names) were commercially available, were used as such without further purification. All the chemicals and reagents used in present investigation were purchased from Sigma Aldrich Chemical Company.

Chemistry:

General Synthetic Procedure: To a solution of 2-methylpropan-2-amine in DCM was added N, N-di-isopropyl ethylamine followed by 3-nitro-benzenesulfonyl chloride 1 and stirred at 0 ºC to result N-tert-butyl-3-nitrobenzenesulfonamide 2. A solution of 2 in 1,4-dioxane and water was added to Ammonium chloride and Zinc lot wise to furnish 3-amino- N- tert- butylbenzenesulfonamide 3. A mixture of 3 and 2, 4-dichloro-5-methylpyrimidine was stirred to afford N-tert-butyl-3-[(2-chloro-5-methylpyrimidin-4-yl) amino] benzenesulfonamide 4.

Treatment of 4 with various aromatic amines in the presence of $Pd_2(dba)_3$ and X-Phos under MW irradiation conditions obtained the corresponding N-tert-butyl-3-{[5-methyl-2-(arylamino)pyrimidin-4-yl]amino}benzenesulfonamides 5 **Scheme 1**.



Ar	Ar
a: C_6H_5	**e:** 2-ClC_6H_4
b: 2-$CH_3C_6H_4$	**f:** 4-BrC_6H_4
c: 3-FC_6H_4	**g:** 2-$NO_2C_6H_4$
d: 4-FC_6H_4	**h:** 3-$NO_2C_6H_4$

SCHEME 1: SYNTHETIC ROUTES TO THE *N-TERT*-BUTYL-3-{[5-METHYL-2-(ARYLAMINO) PYRIMIDIN-4-YL] AMINO} BENZENESULFONAMIDES 5

In this study several combinations of bases, catalysts and ligands employed in several in different solvents **Table 1**, have been screened, and after extensive studies in utilizing the ligands and catalysts, it is noticed that some sterically hindered phosphine ligands are taking longer time period and less efficient. And finally a successful combination for N-arylation of different anilines, *i.e.* combination of Tris (dibenzylidene-acetone) dipalladium (0), X-Phos (lig-1, **Fig. 1**), Cs_2CO_3 (base), in DMF under microwave irradiation (Biotage Microwave, 300 Watt) for 15 min was identified and , was found to the most efficient and faster system. It has been applied the catalyst system at normal conditions, where the reaction time is high and the isolated yields were found to be less efficient than the corresponding microwave reaction (Entry 13, **Table 1**), at ambient temperature the reaction did not work at all (Entry 17, **Table 1**).

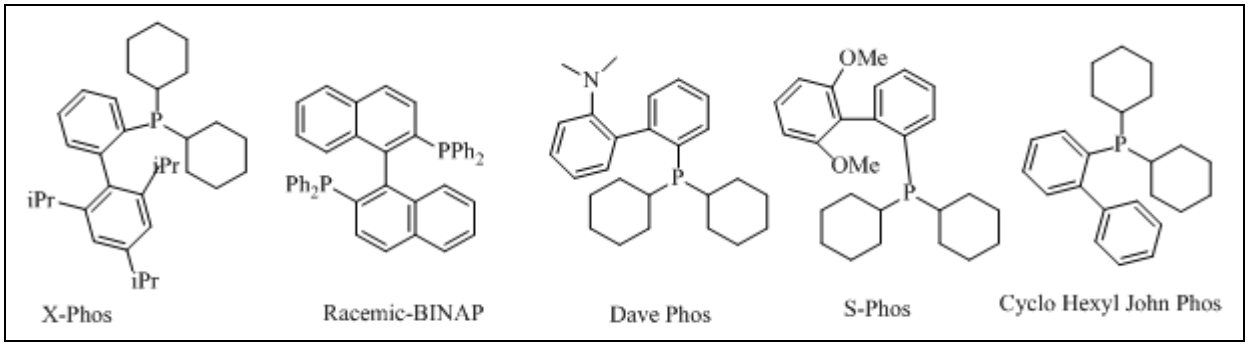


FIG. 1: LIGANDS

TABLE 1: YIELD OF COMPOUNDS WITH LIGANDS

Entry no.	Method (combination)	Reaction time (min)	Temperature (ºC)	*Yield
1	$Pd_2(dba)_3$/X-Phos(lig-1)/Cs_2CO_3/1,4-dioxan	15	150	[a]40-55%
[b]2	$Pd_2(dba)_3$/X-Phos(lig-1)/K_2CO_3/1,4-dioxan	15	150	35%
3	$Pd_2(dba)_3$/X-Phos(lig-1)/Cs_2CO_3/DMF	15	150	[a]80-91%***
[b]4	$Pd(OAc)_2$/BINAP(lig-2)/Cs_2CO_3/DMF	15	150	30%
[b]5	$Pd_2(dba)_3$/Dave Phos(lig-3)/Cs_2CO_3/1,4-dioxan	15	150	45%
[b]6	$Pd_2(dba)_3$/ Dave Phos(lig-3)/K_2CO_3/1,4-dioxan	15	150	40%
7	$Pd_2(dba)_3$/ Dave Phos(lig-3)/Cs_2CO_3/DMF	15	150	[a]50-65%
[b]8	$Pd(OAc)_2$/X-Phos(lig-1)/Cs_2CO_3/DMF	15	150	50%
[b]9	$Pd_2(dba)_3$/S-Phos(lig-4)/Cs_2CO_3/DMF	15	150	60%
[b]10	$PdCl_2$/X-Phos(lig-1)/Cs_2CO_3/DMF	15	150	40%
11	$Pd_2(dba)_3$/JohnPhos(cyclohexyl)(lig-5)/Cs_2CO_3/DMF	15	150	[a]55-70%
[b]12	$Pd_2(dba)_3$/X-Phos(lig-1)/Cs_2CO_3/1,4-dioxan	18h	150	65%**
[b]13	$Pd_2(dba)_3$/X-Phos(lig-1)/Cs_2CO_3/DMF	24h	150	80%**
14	Cs_2CO_3/1,4-dioxan	12h	150	--**
15	Cs_2CO_3/DMF	12h	150	--**
16	K_2CO_3/DMF	12h	150	--**
17	$Pd_2(dba)_3$/X-Phos(lig-1)/Cs_2CO_3/DMF	48h	rt	No reaction
				--**

* Isolated yield after column purification; ** The reactions carried out under normal conditions (not in microwave); ***Method produced better yield; a-The entries screened for all the molecules (5a-h); b-The entries screened only for compound 5b

In a typical case, a mixture of 4, aniline (Ar=C_6H_5) in DMF was added Cs_2CO_3 and Tris (dibenzylidene-acetone) dipalladium (0)/X-Phos at room temperature under argon. The reaction mixture was subjected to microwave irradiation (Biotage Microwave, 300 Watt) at 150 ºC for 15 min. After completion of the reaction as indicated by TLC the reaction mixture was filtered through celite pad and washed with DCM. Filtrate was concentrated under reduced pressure. The obtained crude was purified by silica gel column chromatography (eluted with 5-6% methanol in DCM). All the pure fractions were concentrated to obtain the product 5a.

This reaction was extended to seven other aromatic amines to ascertain the generality of this reaction and products obtained in each case was characterized as *N-tert*-butyl-3-{[5-methyl-2-(aryl-amino)pyrimidin-4-yl]amino}benzenesulfonamides 5b-h **Table 2**.

TABLE 2: YIELD OF COMPOUNDS WITH DIFFERENT SUBSTITUENTS

Compound	Ar	Reaction time (min)	Temperature (ºC)	*Yield
5a	C_6H_5	15	150	90%
5b	2-$CH_3C_6H_4$	15	150	89%
5c	3-FC_6H_5	15	150	80%
5d	4-FC_6H_5	15	150	83%
5e	2-ClC_6H_4	15	150	87%
5f	4-BrC_6H_4	15	150	91%
5g	2-$NO_2C_6H_4$	15	150	81%
5h	3-$NO_2C_6H_4$	15	150	82%

* Isolated yield after column purification

Characterisation of Synthesised Compounds:

N-tert-Butyl-3-nitrobenzenesulfonamide (2): To a solution of 2-methylpropan-2-amine (2.19 g, 0.03 mol) in DCM (15 ml) N,N-di-isopropylethylamine (3.87 g, 0.03 mol) was added, followed by 3-nitrobenzenesulfonyl chloride 1 (2.21 g**,** 0.01 mol) at 0 ºC. The resulting reaction mixture was stirred at room temperature for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with DCM (100 ml). The organic layer was washed with saturated aqueous sodium bicarbonate and brine solution.

The separated organic layer dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure, crude was purified by column chromatography on 100-200 silica gel by eluting with 50% ethyl acetate in n-hexane, obtained pale yellow compound 2, yield: 92%; m.p. 100-102 ºC. IR (KBr) ν_{max} (cm^{-1}): 3288, 3082, 2981, 2873, 1606, 1528, 1469, 1422, 1391, 1324, 1155, 1075; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28(s, 9H, 3XCH_3), 4.62 (brs, 1H, NH), 7.73 (t, 1H, Ar-H, J=7.2Hz), 8.23 (d, 1H, Ar-H, J=7.2Hz), 8.41 (d, 1H, Ar-H, J=7.2Hz), 8.73 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 149.2, 144.2, 129.3, 116.5, 113.3, 111.0, 53.1, 29.8; LC-MS: *m/z* 258.1[M] $^+$.Anal. Calcd for $C_{10}1H_4N_2O_4S$: C 46.50%, H 5.46%, N 10.85%. Found: C 46.63%, H 5.49%, N 10.91%.

3-Amino-N-tert-butylbenzenesulfonamide (3): To a solution of *N-tert*-butyl-3-nitrobenzene-sulfonamide 2 (2.58 g, 0.01 mol) in 1,4-dioxane: water (8:2, 25 ml) was added Ammonium chloride (3.20 g, 0.06 mol) and Zinc (3.9g, 0.06 mol) lot wise at 0 ºC. This reaction mixture was stirred at room temperature for 4.0 h. After completion of reaction as indicated by TLC, the reaction mixture was filtered through celite pad and washed with ethylacetate, the filtrate was saturated with sodium bicarbonate solution. The organic layer was dried over anhydrous Na_2SO_4 and the solid was chromatographed on silica gel to give a white solid 3, yield: 89%; m.p. 122-124 ºC. IR (KBr) ν_{max} (cm^{-1}): 3475, 3378, 3308, 2975, 2944, 1628, 1596, 1530, 1418, 1369, 1308, 1210, 1136, 1082 ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08(s, 9H, 3XCH_3), 5.48 (brs, 2H, NH_2), 6.67 (d, 1H, Ar-H, J=7.4Hz), 6.90 (d, 1H, Ar-H, J=7.4Hz), 7.00 (s, 1H, Ar-H), 7.15 (t, 1H, Ar-H, J=7.4Hz), 7.26 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 147.7, 146.1, 132.6, 131.5, 126.6, 121.0, 53.8, 29.8; LC-MS: *m/z* 228.0[M]$^+$. Anal. Calcd for $C_{10}1H_6N_2O_2S$: C, 52.61; H, 7.06; N, 12.27. Found: C, 52.69; H, 7.08; N, 12.29%.

N- tert- Butyl- 3- [(2-chloro-5-methylpyrimidin-4-yl) amino] benzenesulfonamide (4): To a solution of 2, 4-dichloro-5-methylpyrimidine (1.63 g, 0.01 mol) in methanol was added 3-amino-*N-tert*-butyl benzene sulfonamide 3 (2.28 g, 0.01 mol). The resulting reaction mixture was stirred at 50 ºC for 12 h. On completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature. The obtained product was filtered and purified by recrystallization from ethanol to obtain white solid 4, yield: 84%; m.p. 197-199 ºC. IR (KBr) ν_{max} (cm^{-1}): 3327, 2974, 2944, 2843, 1605, 1560, 1503, 1464, 1433, 1364, 1290, 1223, 1127, 1091; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12(s, 9H, 3XCH_3), 2.18(s, 3H, CH_3), 7.50-7.53 (m, 3H, Ar-H), 7.86 (brs, 1H, NH), 8.10-8.11 (s, 2H, NH, pyrimidine-H), 9.10 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 160.1, 156.5, 144.6, 138.8, 129.1, 124.8, 121.3, 120.2, 114.5, 53.1, 29.6, 13.4; LC-MS: *m/z* 355.1 [M+H]$^+$. Anal. Calcd for $C_{15}1H_9ClN_4O_2S$: C, 50.77; H, 5.40; N, 15.79. Found: 50.87; H, 5.43; N, 15.83%.

N- tert- Butyl- 3- {[5- methyl- 2- (arylamino) pyrimidin-4-yl] amino benzene sulfonamide (5): To a stirred solution of *N-tert*-butyl-3-[(2-chloro-5-methylpyrimidin-4-yl)amino]-benzenesulfonamide 4 (3.54 g, 0.01 mol) and aromatic amine (0.015 mol) in DMF was added Cs_2CO_3 (0.04 mol) at room temperature and degas with argon for 15 min and added Tris (dibenzylidene-acetone) dipalla-dium (0) (4 mol%) and X-Phos (4 mol%) at room temperature under argon. The reaction mixture was subjected to microwave irradiation (Biotage Microwave, 300 Watt) at 150 ºC for 15 min. The reaction mixture was filtered through celite pad and washed with DCM. Filtrate was concentrated under reduced pressure. The obtained crude was purified by silica gel column chromatography (eluted with 5-6% methanol in DCM). All the pure fractions were concentrated to obtain the products 5a-h.

N- tert- Butyl- 3- {[5- methyl- 2- (phenylamino) pyrimidin-4-yl]amino}benzene-sulfonamide (5a): White coloured solid, m.p. 214-216^{o}C. IR (KBr)

ν_{max} (cm^{-1}): 3362, 3326, 3062, 2980, 2862, 1609, 1576, 1529, 1474, 1421, 1295, 1146, 1103; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (s, 9H, 3XCH_3), 2.12 (s, 3H, CH_3), 6.85 (t, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 7.18 (t, 2H, Ar-H), 7.48-7.49 (m, 2H, Ar-H), 7.58 (d, 2H, Ar-H), 7.92 (s, 1H, pyrimidine-H), 8.02 (brs, 1H, NH), 8.08 (s, 1H, Ar-H), 8.52 (brs, 1H, NH), 9.23 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.8, 158.0, 155.7, 144.2, 140.9, 140.1, 128.8. 128.2, 125.0, 120.3, 119.9, 119.2, 118.4, 106.6, 53.1, 30.0, 13.8; LC-MS: *m/z* 412.2 $[M+H]^+$. Anal. Calcd for $C_{21}H_{25}N_5O_2S$: C 61.29%, H 6.12%, N 17.02%. Found: C 61.38%, H 6.14%, N 17.02%.

N-tert-Butyl- 3- {[5-methyl- 2- (2- methylphenyl-amino)pyrimidin-4-yl]amino}-benzenesulfonamide (5b): White coloured solid, m.p. 166-169 ºC; IR (KBr) ν_{max} (cm^{-1}): 3314, 3198, 3061, 2975, 1659, 1597, 1525, 1552, 1478, 1435, 1390, 1298, 1141, 1100; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (s, 9H, 3XCH_3), 2.0 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 7.01 (t, 1H, Ar-H), 7.13-7.18 (dd, 2H, Ar-H), 7.27 (t, 1H, Ar-H), 7.38 (t, 1H, Ar-H), 7.48-7.52 (m, 3H, Ar-H), 7.84 (s, 1H, pyrimidine-H), 8.48 (brs, 1H, NH), 11.9 (brs, 2H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 159.4, 144.7, 140.8, 138.7, 131.4, 130.5, 128.9, 124.5, 123.6, 123.5, 120.0, 118.5, 111.4, 105.4, 52.8, 29.6, 21.9, 13.5; LC-MS: *m/z* 425.0 $[M]^+$. Anal. Calcd for $C_{22}H_{27}N_5O_2S$: C 62.09%, H 6.40%, N 16.46%. Found: C 62.18%, H 6.43%, N 16.51%.

N- tert- Butyl- 3- {[5-methyl-2- (3- fluorophenyl-amino) pyrimidin-4-yl] amino}benzene- sulfonamide (5c): White coloured solid, m.p. 194-195 ºC; IR (KBr) ν_{max} (cm^{-1}): 3314, 3198, 3061, 2975, 1659, 1597, 1525, 1552, 1478, 1435, 1390, 1298, 1141, 1100; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (s, 9H, 3XCH_3), 2.0 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 7.01 (t, 1H, Ar-H), 7.13-7.18 (dd, 2H, Ar-H), 7.27 (t, 1H, Ar-H), 7.38 (t, 1H, Ar-H), 7.48-7.52 (m, 3H, Ar-H), 7.84(s, 1H, pyrimidine-H), 8.48 (brs, 1H, NH), 11.9 (brs, 2H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 159.4, 144.7, 140.8, 138.7, 131.4, 130.5, 128.9, 124.5, 123.6, 123.5, 120.0, 118.5, 111.4, 105.4, 52.8, 29.6, 21.9, 13.5; LC-MS: *m/z* 425.0 $[M]^+$. Anal. Calcd for $C_{21}H_{24}FN_5O_2S$: C 58.72%, H 5.63%, N 16.31%. Found: C 58.84%, H 5.64%, N 16.35%.

N- tert- Butyl- 3- {[5-methyl-2-(4-fluorophenyl-amino) pyrimidin- 4-yl]amino}benzene- sulfonamide (5d): White coloured solid, m.p. 185-187 ºC; IR (KBr) ν_{max} (cm^{-1}): 3245, 3162, 3113, 2980, 2927, 2864, 1660, 1634, 1586, 1537, 1484, 1434, 1323, 1199, 1141, 1087; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (s, 9H, 3XCH_3), 2.12 (s, 3H, CH_3), 7.02 (t, 2H, Ar-H), 7.49 (s, 2H, Ar-H), 7.50 (s, 1H, Ar-H), 7.61-7.63(m, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 8.07 (s, 2H, NH, pyrimidine-H), 8.60 (brs, 1H, NH), 8.99 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.5, 162.1, 150.8, 145.4, 141.7, 138.1, 136.3, 131.5, 129.8, 128.6, 124.0, 122.4, 116.0, 107.0, 53.0, 29.8, 13.5; LC-MS: *m/z* 430.2 $[M+H]^+$. Anal. Calcd for $C_{21}H_{24}FN_5O_2S$: C 58.72%, H 5.63%, N 16.31%. Found: C 58.85%, H 5.65%, N 16.34%.

N- tert- Butyl- 3- {[5-methyl-2-(2-chlorophenyl-amino) pyrimidin-4-yl] amino} benzene-sulfonamide (5e): White coloured solid, m.p. 216-217 ºC. IR (KBr) ν_{max} (cm^{-1}): 3429, 3335, 3207, 3058, 2970, 2864, 1695, 1603, 1580, 1531, 1422, 1284, 1185, 1138, 1086; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (s, 9H, 3XCH_3), 2.15 (s, 3H, CH_3), 6.97-7.03 (m, 2H, Ar-H), 7.50-7.65 (m, 5H, Ar-H), 7.92 (s, 1H, NH), 8.06 (s, 2H, pyrimidine-H, Ar-H), 8.85 (s, 1H, NH), 9.97 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 160.5, 159.3, 157.7, 155.7, 144.9, 143.3, 140.0, 129.4, 129.1, 125.1, 120.5, 118.7, 114.7, 105.9, 105.2, 53.2, 30.5, 13.3;LC-MS: *m/z* 447.0 $[M+H]^+$.Anal. Calcd for $C_{21}H_{24}BrN_5O_2S$: C 51.43%, H 4.93%, N 14.28%. Found: C 51.52%, H 4.96%, N 14.31%.

N- tert- Butyl- 3- {[5-methyl-2-(4-bromophenyl-amino) pyrimidin-4-yl] amino}-benzene-sulfonamide (5f): White coloured solid, m.p. 290-292 ºC; IR (KBr) ν_{max} (cm^{-1}): 3430, 3415, 3083, 3040, 2987, 2882, 1661, 1593, 1575, 1515, 1469, 1410, 1319, 1209, 1121, 1089; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.08 (s, 9H, 3XCH_3), 2.16 (s, 3H, CH_3), 7.38 (s, 4H, Ar-H), 7.61 (t, 2H, Ar-H), 7.71 (d, 1H, Ar-H, J=7.4Hz), 7.86 (d, 1H, Ar-H, J=7.4Hz), 7.92 (s, 2H, NH, pyrimidine-H), 9.73 (brs, 1H, NH), 10.13 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.2, 161.1, 150.2, 148.2, 145.1, 139.2, 140.1, 131.4, 130.0, 129.2, 126.4, 125.3, 123.5, 118.1, 117.2, 105.1, 53.3, 28.9, 14.1; LC-MS: *m/z* 489.9 $[M+H]^+$. Anal. Calcd for

$C_{21}H_{24}FN_5O_2S$: C 58.72%, H 5.63%, N 16.31%. Found: C 58.88%, H 5.64%, N 16.35%.

N- tert-Butyl-3-{[5-methyl-2-(2-nitrophenylamino) pyrimidin-4-yl]amino}-benzenesulfonamide(5g): Yellow coloured solid, m.p. 238-240 ºC; IR (KBr) ν_{max} (cm^{-1}): 3404, 3266, 3190, 3113, 2982, 2870, 1645, 1610, 1532, 1477, 1426, 1301, 1216, 1148, 1096; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18 (s, 9H, 3XCH_3), 2.18 (s, 3H, CH_3), 7.00 (m, 1H, Ar-H), 7.18 (m, 2H, Ar-H), 7.29 (m, 1H, Ar-H), 8.07-8.14 (m, 5H, NH, 4Ar-H), 7. 81 (s, 1H, pyrimidine-H), 8.55 (brs, 1H, NH), 10.12 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.7, 157.9, 155.9, 149.1, 143.8, 142.2, 140.5, 129.5, 129.1, 125.1, 124.3, 120.3, 119.1, 114.5, 111.9, 107.7, 53.2, 29.7, 14.0; LC-MS: *m/z* 456 $[M]^+$. Anal. Calcd for $C_{21}H_{24}N_6O_4S$: C 55.25%, H 5.30%, N 18.41%. Found: C 55.34%, H 5.32%, N 18.48%.

N- tert- Butyl- 3- {[5-methyl-2-(3-nitrophenyl-amino) pyrimidin-4-yl]amino}- benzenesulfonamide (5h): Yellow coloured solid, m.p. 190-191 ºC; IR (KBr) ν_{max} (cm^{-1}): 3348, 3317, 2984, 2868, 1681, 1646, 1595, 1559, 1502, 1419, 1309, 1146, 1108 ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (s, 9H, 3XCH_3), 2.15 (s, 3H, CH_3), 7.49-7.54(m, 4H, Ar-H), 7.69 (brs, 1H, NH), 8.04 (m, 3H, pyrimidine-H, 2Ar-H), 8.15 (brs, 1H, NH), 8.67 (d, 2H, Ar-H), 9.53 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 162.6, 156.3, 147.5, 146.1, 144.0, 141.4, 140.1, 139.4, 129.1, 125.0, 121.2, 119.8, 119.1, 116.6, 101.9, 50.5, 30.5, 13.3; LC-MS: *m/z* 457.0 $[M+H]^+$. Anal. Calcd for $C_{21}H_{24}N_6O_4S$: C 55.25%, H 5.30%, N 18.41%. Found: C 55.36%, H 5.34%, N 18.49%.

RESULTS AND DISCUSSION: In the present study it was observed a considerable progress in the modification of Buchwald-Hartwig reactions, by an efficient catalyst system for the synthesis of pyrimidinyl sulfonamides under microwave (Biotage Microwave, 300 Watt) conditions with best results.Compound 2 displayed a characteristic absorption band in the IR spectra at 3395 cm^{-1} due to NH group. The ^{1}H NMR spectrum exhibited a signal at δ 4.62 ppm as a broad singlet integrating one proton which corresponds to one NH proton. The mass spectrum of the product 2 also agrees with the structure displayed (M^+) ion peak at m/z 258.1.

The structure of compound 3 was identified by its two characteristic absorption bands in the IR spectra at 3475, 3308 cm^{-1} due to NH_2 group and did not display absorption bands due to $–NO_2$ functional group present in its precursor 2, confirming the formation of compound 3. The ^{1}H NMR spectrum displayed a broad singlet with integrating two protons at δ 5.48 ppm corresponds to the NH_2 protons. The mass spectrum of the product 3 also agrees the structure showed (M^+) ion peak at m/z 228.0.

Development of the compound 4 was identified by its IR band at 3327 cm^{-1} due to NH and did not display absorption bands due to $–NH_2$ functional group present in its precursor 3. The ^{1}H NMR spectrum exhibited a singlet at 8.10-8.11 which corresponding to NH and pyrimidine proton and did not display signals due to $–NH_2$ protons present in its precursor 3. The mass spectrum of 4 confirmed the structure by exhibiting $[M+H]^+$ ion peak at m/z 355.1.

Emergence of the compound 5 was established by the study of different spectra. The IR spectrum of the compound 5a showed two characteristic absorption bands at 3362 and 3326 cm^{-1} due to the NH functional groups and did not display single absorption band due to NH functional group present in its precursor 4, confirming the formation of the compound 5a. Similarly, the formation of 5a was supported by the ^{1}H NMR spectrum exhibited a signal with one proton at δ 8.52 ppm as a broad singlet which corresponds to the NH proton that was absent in its precursor 4. The mass spectrum of the product 5a also agrees with the structure displayed $[M+H]^+$ ion peak at m/z 412.2. The chemical structures of the remaining compounds 5b-h were identified with the same protocol.

The structures of the products 2-5 have been elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. Elemental analyses were satisfactory and confirm elemental composition and purity of newly synthesized compounds 2-5.

Antibacterial Activity: *In-vitro* screening of antibacterial activities of 5a-5h in dimethylsulfoxide (DMSO) were performed by the broth dilution method using nutrient agar against Gram-negative bacteria *Pseudomonas aeruginosa*,

Klebsiella aerogenes, *Chromobacterium violaceum*, and Gram-positive bacteria *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus* at 100 µg/ml concentration. The minimum inhibitory concentration (MIC) was done by the broth dilution method (24). The ready-made nutrient broth medium (HiMedia, 25 g) was suspended in distilled water (100 ml) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc^2 for 25 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound is dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 µg/ml and added to the first test tube, which was serially diluted. A fixed 0.5 ml volume of overnight culture is added to all the test tubes and then incubated at 35 ºC for 24 h. After 24 h, these tubes were measured for turbidity. Ciprofloxacin and Trimethoprim were used as standards for comparison. Results are given in **Table 3**.

The results of antibacterial screening reveal that compounds 5a-5h displayed good activity. The compounds 5d and 5e possessing fluoro and chloro groups as substituent on the benzene ring exhibited a better activity. However, the degree of inhibition varied both with test compound as well as with the bacteria used in the present investigation. In conclusion, almost all the series of compounds 5a-5h showed good activity by inhibiting growth of all the bacteria to a greater extent. These remarkable results may be due to the presence of the pyrimidine ring linked to sulfonamide group. Some of the compounds may be used as bacteriocides after a detailed study.

TABLE 3: ANTIBACTERIAL ACTIVITY OF 5a-5h

	MIC[a,b]					
	Gram-positive			Gram-negative		
Compound	*B. substilis*	*B. sphaerius*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
5a	25	28	27	37	29	31
5b	20	26	24	30	27	25
5c	24	25	30	35	26	27
5d	19	21	18	31	22	24
5e	17	20	19	28	25	23
5f	21	29	22	30	28	29
5g	23	27	20	37	26	26
5h	22	26	27	33	27	27
Ciproflaxacin	20	25	20	30	25	25
Trimethoprim	21	23	21	28	22	25

Notes: [a]Negative control (DMSO)-no activity; [b]Concentration 100 µg/ml.

Antifungal Activity: Antifungal activities of 5a-5h were determined by using the agar cup bioassay method (25) with Clotrimazole as the standard. The compounds were tested for their antifungal activity against five test organisms, *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae, Fusarium moniliforme* and *Curvularia lunata* using the agar cup bioassay method at 100µg/ml concentrations.

The ready-made nutrient broth medium (HiMedia, 40 g) was suspended in distilled water (1000 ml) and heated until it dissolved completely. The medium and petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. The medium was poured into sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of culture of the test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in dimethylsulfoxide (DMSO) at a concentration of 100 µg/ml. Agar inoculation cups were scooped out with a 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, (100 µg/ml) of the test solution was added. Controls were maintained with DMSO and Clotrimazole (100 µg/ml). The treated and controls were kept at room temperature for 72-95 h. Inhibition zones were determined and diameter was calculated in millimetre. Three to four replicates were maintained for each treatment. The results were given in **Table 4**.

The antifungal activity results indicated that these compounds 5a-5h were significantly toxic towards all five fungi and they were lethal even at 100 µg/ml concentration. In series 5, compounds 5e and 5f exhibited high antifungal activity which may be

due to the presence of chloro, bromo groups as substituents on the benzene ring. The antifungal activity of these compounds compared with the standard drugs Clotrimazole and Fluconazole, which demonstrated that they have promising activity. In conclusion, almost all the series of compounds 5a-5h are moderately toxic towards the fungi under investigation and they were lethal even at 100 µg/ml concentration in comparison with standard Clotrimazole and Fluconazole at the same concentration. This may be due to the presence of pyrimidine ring linked to sulfonamide group.

TABLE 4: ANTIFUNGAL ACTIVITY OF 5a-5h

	Zone of inhibition[a,b]				
Compound	***A. niger***	***C. tropicum***	***R. oryzae***	***F. moniliformae***	***C. lunata***
5a	25	22	20	18	24
5b	28	22	21	19	22
5c	27	24	21	17	25
5d	24	21	19	16	21
5e	28	26	21	19	20
5f	29	24	22	18	25
5g	23	21	17	14	18
5h	22	24	14	17	21
Clotrimazole	30	29	23	20	28
Fluconazole	28	30	27	24	30

Notes: [a]Negative control (DMSO)-no activity; [b]Concentration 100 µg/ml.

CONCLUSION: A new efficient catalyst/ligand combination was developed for the synthesis of title compounds 5a-5h under microwave conditions. The microwave procedure was slightly superior than the conventional method in terms of reduced time period and better yields. Among the title compounds 5d and 5e exhibited potent activity towards both gram positive and gram negative bacteria. Compounds 5e and 5f showed good antifungal activity.

ACKNOWLEDGMENT: We, the authors, express our sincere gratitude to management, J.K.C. College, Guntur, for providing workspace.

CONFLICT OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

REFERENCES:

1. Canals A, Arribas-Bosacoma R, Albericio F, Álvarez M, Aymamí J and Colla M: Intercalative DNA binding of the marine anticancer drug variolin B. Scientific reports 2017; 7: 39680.
2. Yunpeng Y, Ximing X, Yu L, Shuijin X and Ruofeng S: Synthesis and antibacterial activities of novel pleuromutilin derivatives with a substituted pyrimidine moiety. Eur. J. Med. Chem 2017; 126: 687-695.
3. Giles D, Roopa K, Sheeba FT, Gurubasavarajaswamy PM, Divakar G and Vidhya T: Synthesis pharmacological evaluation and docking studies of pyrimidine derivatives. Eur. J. Med. Chem 2012; 58: 478-484.
4. Tatjana GK, Natasa I, Visnja S, Lana S, Jasna P, Kraljevic, Sandra P, and Raic-Malic S: Synthesis and in vitro antiproliferative evaluation of novel N-alkylated 6-isobutyl- and propyl pyrimidine derivatives. Bioorganic & Medicinal Chemistry Letters 2014; 13: 2913-2917.
5. Fargualy AM, Habib NS, Ismail KA, Hassan AMM and Sarg MTM: Synthesis, biological evaluation and molecular docking studies of some pyrimidine derivatives: European Journal of Medicinal Chemistry 2013; 66: 276-295.
6. Suryawanshi SN, Kumar S, Shivahare R, Pandey S, Tiwari A and Gupta S: Design, synthesis and biological evaluation of aryl pyrimidine derivatives as potential leishmanicidal agents: Bioorg. Med. Chem. Lett 2013; 23: 5235-5238.
7. Soni HI and NB: Pyrimidine incorporated Schiff base of isoniazid with their synthesis, characterization and *in-vitro* biological evaluation. Asian Journal of Pharmaceutical and Clinical Research 2017; 10(10): 209.
8. Mohana KN, Basavapatna N, Kumar P and Lingappa Mallesha L: Synthesis and biological activity of some pyrimidine derivatives.Drug Invention Today 2013; 5(3): 216-222.
9. Al-Adiwish WM, Tahir MIM, Siti-Noor-Adnalizawati A, Hashimc SF, Ibrahim N and Yaacob WA: Synthesis, antibacterial activity and cytotoxicity of new fused pyrazolo[1,5-a]pyrimidineand pyrazolo[5,1-c][1,2,4] triazine derivatives from new 5-aminopyrazoles. Eur J Med Chem 2013; 64: 464-476.
10. Bhuva NH, Talpara PK, Singala PM, Gothaliya VK and Shah VH: Synthesis and biological evaluation of pyrimidinyl sulphonamide derivatives as promising class of antitubercular agents. Journal of Saudi Chemical Society 2017; 21: 517-527.
11. Rani J, Saini M, Kumar S and Verma PK: Design, synthesis and biological potentials of novel tetrahydroimidazo[1,2-a]pyrimidine derivatives.Chemistry central journal December 2017; 11: 16.
12. Bhat AR, Dongre RS, Naikoo GA,Hassan IU and Ara T: Proficient synthesis of bioactive annulated pyrimidine derivatives: A review. Journal of Taibah University of Science November 2017; 11(6): 1047-1069.
13. Vembu S and Gopalakrishnan M: Synthesis, characterization and antimicrobial screening of novel bis-2-aminopyrimidines.Journal of Pharmacy Research 2014; 8(4): 548-551.

14. Mohammed HH, Nasif ZN, Mageed ZN and Ahmed S:S ynthesis and biological activities of new fused pyrimidine compound. Asian Journal of Chemistry 2018; 30(3):661-666.
15. Devender N, Gunjan S, Tripathi R and Tripathi R: Synthesis and antiplasmodial activity of novel indoleamide derivatives bearing sulfonamide and triazole pharmacophores. Eur. J.Med. Chem 2017; 131: 171-184.
16. Fares M, Eladwy RA, Nocentini A, Abd-El-HadiSR, Ghabbour HA, Megeed AA, Eldehna WM, Abdel-Aziz HA and Supuran CT: Synthesis of bulky-tailed sulfonamides incorporating pyrido[2,3-d][1,2,4]triazolo [4,3-a]pyrimidin-1(5H)-yl) moieties and evaluation of their carbonic anhydrases I, II, IV and IX inhibitory effects.Bioorg. Med. Chem 2017; 25: 2210-2217.
17. Ramazan U, Kurt BZ, Gazioglu I and Muharrem K: Microwave assisted synthesis of novel hybrid tacrine-sulfonamide derivatives and investigation of their antioxidant and anticholinesterase activities. Bioorg. Chem 2017; 70: 245-255.
18. Sun L, Wu Y, Liu Y, Chen X and Hu L: Novel carbazole sulfonamide derivatives of antitumor agent. Synthesis, antiproliferative activity and aqueous solubility. Bioorg. Med. Chem Lett 2017; 27: 261-265.
19. Santagada V, Frecentese F, Perissutti E, Favretto L and Caliendo G: The application of microwaves in combinatorial and high-throughput synthesis as new synthetic procedure in drug discovery. QSAR & Combinatorial Science 2004; 23(10): 919-944.
20. Fahim AM: Regioselective synthesis of novel fused sulphonamide derivatives utilizing microwave irradiation. Current Microwave Chemistry 2018; 5: 4-12.
21. Majumder A, Gupta R and Jain A: Microwave-assisted synthesis of nitrogen-containing heterocycles. Green chemistry letters and reviews 2013; 6(2): 151-182.
22. Jainey PJ and Bhat KI: Microwave assisted synthesis of novel pyrimidines bearing benzene sulfonamides and evaluation of anticancer and antioxidant activities. Asian J Pharm Clin Res 2014; l 7(1): 111-114.

How to cite this article:
Nannapaneni M and Boggavarapu J: Synthesis and biological screening of pyrimidine linked benzene sulfonamide derivatives. Int J Pharm Sci & Res 2018; 9(12): 5534-43. doi: 10.13040/IJPSR.0975-8232.9(12).5534-43.