Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(2): 134-136

Research Article ISSN 0975-248X

Synthesis of N-[4-({4-[(5-methyl-1, 3, 4-thiadiazol-2-yl)sulfamoyl] phenyl}sulfamoyl)phenyl]amine: An Impurity in the Antibacterial Drug Sulfamethizole

G B Talagadadeevi, B R Vancha, S Anusuri, M. Rao. Jampani*

R & D Department, Posh Chemicals Pvt. Ltd. 86/C phase-1 IDA, Jeedimetla, Hyderabad, Andhra Pradesh, India

ABSTRACT
The process for the preparation of antibacterial compound 4-amino-N-(5-methyl-1, 3, 4-thiadiazol-2-yl)benzenesulfonamide (Sulfamethizole) **4** involves the reaction of 2-amino-5-methyl-1, 3, 4-thiadiazole **1** with 4-acetamidobenzenesulfonyl chloride **2** followed by alkaline hydrolysis of the intermediate N-{4-[(5-methyl-1, 3, 4-thiadiazol-2-yl)sulfamoyl]phenyl}acetamide **3**. The HPLC analysis of sulfamethizole showed the formation of an impurity which was isolated and identified as N-[4-({4-[(5-methyl-1, 3, 4-thiadiazol-2-yl) sulfamoyl] phenyl} sulfamoyl) phenyl] amine **6**. This impurity is the result of the reaction of unreacted sulfonyl chloride **2** with sulfamethizole to form N-[4-({4-[(5-methyl-1, 3, 4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl]acetamide **5** followed by alkaline hydrolysis of the acetamido group. The structure was further confirmed by independent synthesis.

Keywords: Sulfamethizole, antibacterial, 4-sulfanilinomyl sulfamethizole, HPLC analysis.

INTRODUCTION

The investigations into the pharmacological activity [1-9] of sulfanilamide derivatives of some heterocyclic compounds led to the discovery of 4-amino-N-(5-methyl-1, 3, 4-thiadiazol-2-yl)benzenesulfonamide **4** (sulfamethizole) as an antibacterial used for the treatment of urinary tract infection. [10] There are several literature reports on the synthesis [11-18] of sulfamethizole. The widely adapted method involves two steps in which 4-acetamidobenzenesulfonyl chloride **2** is reacted with 2-amino-5-methyl-1, 3, 4-thiadiazole followed by alkaline hydrolysis of the acetamide group (see scheme).
The HPLC analysis of sulfamethizole **4** showed the presence of an impurity. The present investigations involve the identification of the impurity formed in the process and provide a synthetic method for its preparation so as to quantify its content in sulfamethizole.

MATERIALS AND METHODS

Commercially available reagent grade chemicals were used as received. The reactions were monitored by TLC on E. Merck DC Kieselgel 60 F_{254} coated aluminum sheets. Melting points were determined by capillary method on a Thermonik melting point apparatus model C PMB-2 and are uncorrected. The ^{1}HNMR spectrum was recorded on Bruker model av 400 instrument. Chemical shifts are reported in δ ppm relative to TMS. The ESI mass spectra were recorded on Applied Biosystems model
API 2000 /MDS sciex spectrometer.

Analytical HPLC: The HPLC analysis was carried out on Jasco model 2089 with a PDA detector.
Column: Thermo BDS – C18 (250 mm × 4.6 mm, 5µ)
Sample preparation: A solvent mixture containing methanol: water (85:15) was used in the preparation of the sample for injection. 20 mg of sulfamethizole containing 22% of the impurity was taken in 10 ml of solvent mixture and sonicated to dissolve completely and made up to 25 ml. This solution was further diluted by taking 1 ml and diluting to 1000 ml with solvent mixture of methanol: water (85:15).
Injection volume: 20µl
Flow rate: 1.0 ml/min as per the gradient given in the table below
Mobile phase A: water: methanol: acetic acid (8.5:1.4:0.1)
Mobile phase B: methanol: acetic acid (9.9:0.1)
Table Program: gradient for HPLC analysis

Analytical HPLC

Time (min)	Mobile phase	
	%A	%B
0	100	0
30	100	0
50	30	70
55	30	70
57	100	0

***Corresponding author: Mr. M. Rao. Jampani,** R & D Department, Posh Chemicals Pvt. Ltd, 86/C phase-1 IDA, Jeedimetla, Hyderabad- 500055, Andhra Pradesh, India;
E-mail: poshchemrnd@yahoo.com

Preparative HPLC

Time (min)	Mobile phase	
	%A	%B
0	90	10
10	50	50
14	50	50
16	90	10
20	90	10

Preparative HPLC: The prep analytical work was carried out on a Jasco-2087 plus model with MD2070 plus detector.
Column: Inertsil Prep. ODS – C18 (250 mm × 20 mm, 10µ)
Sample preparation: 250 mg of sulfamethizole containing 22% of the impurity was dissolved in 15 ml of methanol and sonicated to dissolve completely. The solution is diluted with 10 ml of water.
Injection volume: 1 ml
Flow rate: 8.0 ml/min as per the gradient given in the table above
Mobile phase A: water: methanol: acetic acid (7:3:0.01)
Mobile phase B: methanol

Isolation of impurity

A mixture consisting of 200 g technical grade sulfamethizole containing 0.6% of the impurity and 800 ml MeOH was refluxed for 1hr cooled to RT and filtered under suction. The filtrate was concentrated to dryness to get a solid residue (40 g). The analysis indicated the enrichment of impurity by 5%. The above residue was extracted repeatedly 4 times each time with 40 ml methanol. The combined extracts were concentrated to dryness to give 5 g of a sulfamethizole containing 22% of the impurity. This was further subjected to preparative HPLC and the impurity was isolated in 99% pure state.

N-[4-({4-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl]acetamide 5

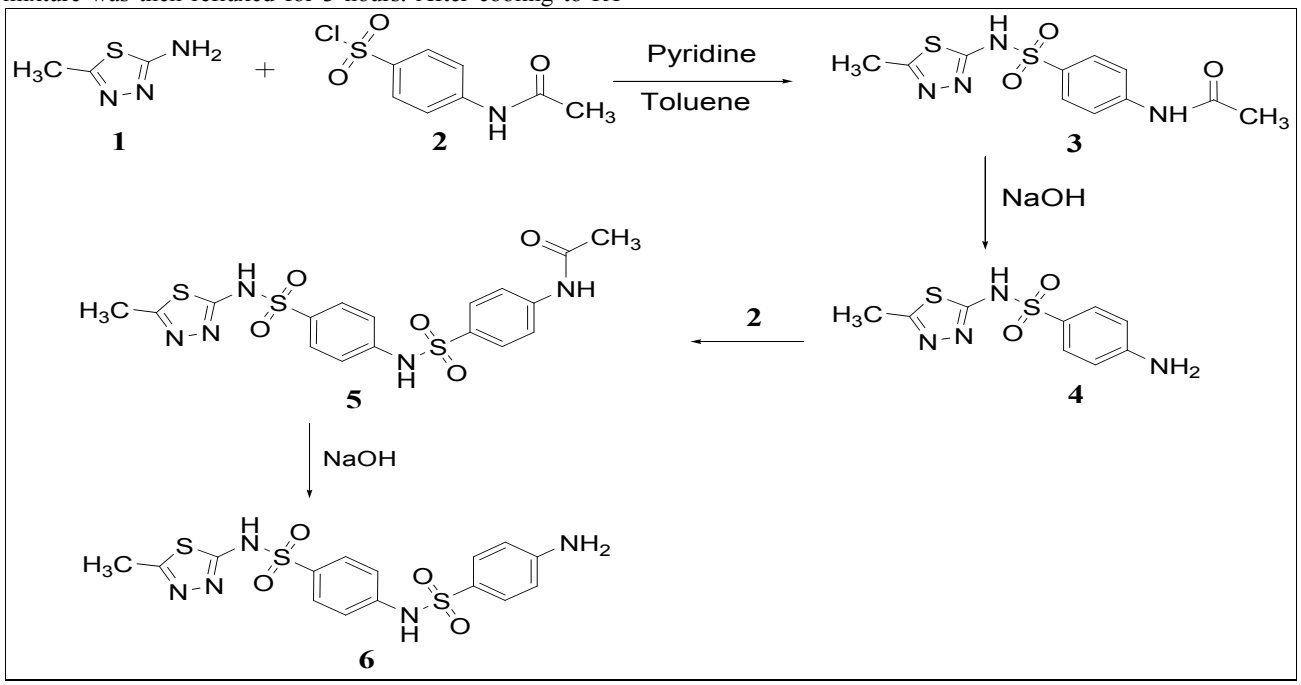
Sulfamethizole **4** (10 g, 0.37 mmol) was suspended in a mixture of toluene (28 ml) and pyridine (6.9 ml). The mixture was cooled to 0-5°C under stirring. Then 4-acetamidobenzenesulfonyl chloride **2** (9 g, 0.38 mmol) was added portion wise over a period of 10 min. The reaction mixture was then refluxed for 3 hours. After cooling to RT 30 ml D.M water was added. The resulting sticky heterogeneous mass was stirred for 30 min, filtered, washed with D.M water (30 ml) and dried to get crude product (8 g). The compound was purified by recrystallization from a mixture of ethyl acetate: methanol (9:1) to give a white solid. m.p. 284°C. Yield: 5.3 g, (30.6%) ^{1}HNMR (DMSO-d_6) δ_{ppm} 2.2 (s, 3H), 2.4 (s, 3H), 7.18-7.2 (d, 2H), 7.61-7.63 (d, 2H), 7.69-7.75 (m, 4H), 10.31 (d, 1H), 10.75 (s, 1H), 13.83 (s, 1H). MS-ESI: 468.0 [M + 1] $^+$ (75), 425.8 (80), 353.3 (100), 310.9 (21), 225.0 (14), 156.0 (20), 116.0 (27).

N-[4-({4-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl]amine 6

N-[4-({4-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl]acetamide **5** (5 g) was dissolved in 5% NaOH (50 ml). The solution was heated and maintained at 100°C for 4 hours under stirring. The solution was cooled to RT and the pH was adjusted to 6 using 30% H_2SO_4. The white solid formed was filtered, washed free from acid and dried. Yield 4 g (92%). This was further purified by preparative HPLC to get white crystalline powder. m.p. 160-161°C. ^{1}HNMR (DMSO-d_6:) δ_{ppm} 2.36 (s, 3H), 6.52-6.54 (d, 2H), 7.15-7.17 (d, 2H), 7.42-7.44 (d, 2H), 7.59-7.61 (d, 2H), three D_2O exchangeable broad singlets at 6.04 (s, 2H) 10.44 (s, 1H), 13.84 (s, 1H) Mass spectrum exhibited highest mass peak corresponding to M+1 at m/e 426.1 (15), and the fragment ions appear at m/e 311.0 (100), 247.1 (76), 156.0 (89), 116.0 (10).Elemental analysis. Found: C, 40.62; H, 3.86; N, 15.79; O, 18.04; S, 21.69.Calcd.for $C_{15}H_{15}N_5O_4S_3$ H_2O: C, 40.53; H, 3.85; N, 15.55; S, 21.39.

RESULTS AND DISCUSSION

The HPLC analysis of technical grade sulfamethizole showed the presence of an impurity eluting at R_t 43.0 min with an area % 0.6. Under preparative HPLC conditions this impurity elutes at R_t 14 min. The mass spectrum of the impurity isolated from sulfamethizole gave highest mass peak at m/e 426.1 corresponding to the molecular formula $C_{15}H_{15}N_5O_4S_3$ indicating the presence of an extra sulfamide function as compared to sulfamethizole. The ^{1}HNMR spectrum showed 8



Scheme

signals total integrating for 15 protons. Of these a singlet at ppm 2.36 integrating for 3 protons is assignable to the methyl group in the thiadiazole moiety present in this compound. A more diagnostic feature is the appearance of three D_2O exchangeable broad singlets at 6.04 (s, 2H) 10.44 (s, 1H), 13.84 (s, 1H) assignable to the protons on the free amino and amide functions. There are 4 doublets each integrating for 2 protons and their multiplicity is typical due to the presence of para disubstituted benzene rings in the compound. Based on this data the compound was identified as (N-[4-({4-[(5-methyl-1, 3, 4-thiadiazol-+2-yl) sulfamoyl] phenyl} sulfamoyl) phenyl] amine **6.** Unambiguous proof for the structure was provided by independent synthesis as depicted in scheme above.

In the first step sulfamethizole **4** was reacted with 4-acetamidobenzenesulphonyl chloride **2** to give N-[4-({4-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl]acetamide **5** which was isolated in pure state and structure established based on spectral data. Compound **5** was then hydrolysed to give **6** and was isolated as monohydrate. The ^{1}HNMR spectrum of the independently synthesized compound was superimposable on that of the impurity isolated from sulfamethizole by preparative HPLC.

The most widely adapted process for the production of sulfamethizole involves reaction of 4-acetamidobenzenesulfonyl chloride **2** with 2-amino-5-methyl-1, 3, 4-thiadiazole **1**. This step however does not result in quantitative yields resulting in the availability of unreacted 2 for side reactions to occur. This gives rise to the formation of process impurities which need to be identified to meet the regulatory prescriptions. Using HPLC methods the process impurity in sulfamethizole was isolated and identified as N-[4-({4-[(5-methyl-1, 3, 4-thiadiazol-2-yl)sulfamoyl]phenyl}sulfamoyl)phenyl] amine **6**. The structure assigned was confirmed by independent synthesis thus providing a route to get the pure compound necessary for the purpose of quantification of its content in the drug.

ACKNOWLEDGEMENT

The authors wish to thank C. S. Ratnaprasad, Managing Director, Posh Chemicals Pvt Ltd for his interest and encouragement.

REFERENCES

1. Ganapathi K. Chemotherapy of bacterial infections Part IV. Synthesis of (N^1)-sulfonamide substituted heterocyclic derivatives of sulfanilamide. Proceedings of the Indian Academy of Sciences Section A 1941; 13 (5): 386-389.
2. Seelemann M. Bacteriostatic effect of newer sulfonamides on streptococcus of different serological groupings. Deut. Tierarztl. Wochenschr. 1942; 50: 221-226.
3. Rune Frisk A. Quantitative chemotherapeutic utilization of 13 N-heterocyclic derivatives of sulfanilamide *in-vitro*. Acta Med. Scand. 1942; 110: 337-358.
4. Tappi G, Migliardi C. Several heterocyclic derivatives of sulfanilamide. Arch. Sci. biol. 1941; 27: 170-175.
5. Rune Frisk A. Sulfanilamides Chemotherapeutic evaluation of N^1-Substituted sulfanilamides. Acta Med. Scand. 1943; 142: 1-199.
6. Guha PC, Dasgupta DB. Sulfanilamide derivatives possessing heterocyclic rings. Sulfanilamidothiadiazoles. Current Sci. 1943; 12: 220.
7. Guha PC, Dasgupta DB. Sulfanilamides I. N^1- Thiadiazolyl sulfanilamides. J. Indian Chem. Soc. 1945; 22: 79-81.
8. Tappi G, Migliardi C. Several sulfanilamide derivatives. Arch. Sci. Biol. 1942; 28: 472.
9. Hartmann M, Cueni F, Druey J, Meyenburg HV. Sulfonamides. US 1942; 2, 386, 852.
10. Peddie BA, Little PJ. Sulphamethizole in urinary tract infections. J. Antimicrob. Chemother. 1979; 5(2): 195-200.
11. Tappi G. Heterocyclic derivatives of sulfanilamide. Recl. Trav. Chim. 1943; 62: 207-209.
12. Roblin Jr RO, Winnek PS. Substituted aryl sulfamidothiadiazoles. US 1944; 2, 358, 31.
13. Kinberg T, Aktiebolaget H. p-Substituted phenyl sulfonamido-1, 3, 4-thiadiazole compounds. GB 1944; 564, 92.
14. Soc. Pour L'ind. Chim. a Bale. Sulfonamide derivatives. CH 1944; 230, 859.
15. Mayer R, Viaud P, Appell F. p-Aminophenylsulfonamidothiadiazoles. Fr. 1941; 866, 175.
16. Hans W, Hildbert W. Synthesis of aminothiadiazoles and the corresponding sulfonamide derivatives. Arch. Pharma. 1951; 284: 53-62.
17. Masaki O, Mifune A, Higashijima T, Nagato S. 1, 3, 4-Thiadiazolederivatives. Several reactions of aminothiadiazoles. J. Pharma. Soc. Japan 1951; 71: 481-484.
18. Ishikawa M, Kikkawa I. Sulfathiadiazole derivatives. Ann. Rept. Shionogi Res. Lab. 1954; 4: 36-38.