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Microwave assisted synthesis of triazolothiadiazole analogues as anticancer and antibacterial agents

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ABSTRACT

*A series of 3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(substituted)[1,2,4]-triazolo[3,4-b][1,3,4]-thiadiazole (**3a-j**) were synthesized by conventional and microwave irradiation methods. Microwave method proved to be a rapid and better yield compare to that of conventional method. The structures of these compounds were established on the basis of spectral and analytical data. These novel compounds were screened for their antibacterial and anticancer activity. The promising compounds **3b** and **3g** have been identified.*

Key words: 1,2,4-Triazoles; Triazolothiadiazoles; Antibacterial activity; Anticancer activity; Microwave irradiation

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INTRODUCTION

Cancer remains the leading cause of death in the world and as a result there is a pressing need for novel and effective treatments. Similarly the problem of multidrug resistant microorganisms has reached on alarming level around the world, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. Therefore the search of novel antibacterial and anticancer agents devoid of side effects continues to be an active area of research in medicinal chemistry.

Designing new drug is based on the development of hybrid molecules by combining different pharmacophore fragments in a single structure, which may lead to compounds with interesting biological profiles.

The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. For example, a traizolo-thiadiazole system may be viewed as a cyclic analog of two important components: thiosemicarbazide and biguanide [1], which often display antibacterial [2], antifungal [3], analgesic [4], anti-inflammatory [5], antitubercular [6], anticancer [7, 8] activities. Fluorinated heterocycles have attracted attention due to the ability of fluorine to act as polar hydrogen or hydroxyl mimic. Therefore substitution of hydrogen by fluorine has been a strategy in designing molecules for biological activity studies [9]. Microwave-assisted reactions dominate over usual conventional methods due to its simplicity in operation, rapid and high yielding of variety of heterocyclic compounds [4]. The application of microwave irradiation to organic synthesis has been the focus of considerable attention in recent years and is becoming an increasingly popular technology [10, 11].

Prompted by these observations, and in continuation of our work on biologically potent heterocyles [12] we planned to synthesize a hitherto unreported novel series of 3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(substituted)[1,2,4]-triazolo[3,4-*b*][1,3,4]-thiadiazole, in order to study their antibacterial and anticancer activity.

MATERIALS AND METHODS

Chemistry

Thin layer chromatography was used to analyze the reaction progress and purity of the compounds synthesized. Melting points were determined by open glass capillary method and were uncorrected. Elemental analysis was carried out in Vario EL III Elemental model. IR spectra were obtained in KBr discs on a Shimadzu-8400 FTIR spectrophotometer, ^{1}H NMR spectra were recorded on Brucker spectrometer (400 MHz) in DMSO-d_6/$CDCl_3$ using TMS as an internal standard, ^{13}C NMR spectra were recorded on Brucker spectrometer (100 MHz) in DMSO-d_6/$CDCl_3$. ^{19}F NMR spectra were recorded on 376 MHz in $CDCl_3$ as solvent, mass spectra were recorded by Agilent 6320 Ion Trap method. Microwave reactions were carried out in Biotage Initiator (400 Watt) microwave synthesizer.

Procedure for synthesis of 4-amino-5-(5'-fluoro-2'-methoxybiphenyl-3-yl)-4H-1,2,4-triazole-3-thiol (2)

To a solution of **1** (66.22 mmol) in ethyl alcohol (200 mL), hydrazine hydrate (231.7 mmol) was added and resulting mixture was heated under reflux for 6 h. The mixture was cooled, diluted with water and acidified with 3N hydrochloric acid. The resulting solid was filtered, washed with water, dried and recrystallized from ethyl alcohol to afford compound **2**.

IR (KBr): 3225, 3143 (NH_2), 1600 (C=N), 2531(SH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.68 (bs, 2H, NH_2), 3.78 (s, 3H, OCH_3), 7.13-7.23 (m, 3H, Ar-H), 7.55-7.61 (m, 2H, Ar-H), 8.21 (d, 1H, *J* = 9.1 Hz, Ar-H), 8.35 (s, 1H, Ar-H), 13.6 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 55.2, 109.9, 111.2, 112.3, 114.7, 117.1, 117.4, 125.2, 127.2, 128.6, 131.4, 151.2, 155.8 (d, *J* = 237.2 Hz), 158.3, 167.3; ^{19}F NMR ($CDCl_3$) δ: -122.3 (m, 1F); LCMS M+1: 317.

General procedure for synthesis of 3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(substituted)[1,2,4]-triazolo[3,4-b][1,3,4]-thiadiazole (3a-j)

Conventional method

A mixture of **2** (1.58 mmol), dry phosphorous oxychloride (4 mL) and carboxylic acid (1.58 mmol) were heated under reflux for 7 h. The resulting reaction mass was poured into crushed ice with stirring. Finally powdered sodium carbonate was added portion wise till the pH of the mixture was raised to 8. The resulting solid was filtered, washed thoroughly with cold water, dried and recrystallized from ethyl alcohol to afford compounds **3a-j**.

Microwave method

A mixture of **2** (1.58 mmol), dry phosphorous oxychloride (1 mL) and carboxylic acid (1.58 mmol) were subjected to MW irradiation (70 W) at 50^{0}C for 5 min. The resulting reaction mass was poured into crushed ice with stirring. Finally powdered sodium carbonate was added portion wise till the pH of the mixture was raised to 8. The resulting solid was filtered, washed thoroughly with cold water, dried and recrystallized from ethyl alcohol to afford compounds **3a-j**.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(2-fluorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole (**3a**)*

IR (KBr): 1587 (C=N), 1248 (N-N=C), 690 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H, -OCH_3), 6.92-6.94 (m, 1H, Ar-H), 7.01-7.15 (m, 2H, Ar-H), 7.22 (dd, 1H, *J* = 8.1, 1.2 Hz, Ar-H), 7.43 (dd, 1H, *J* = 8.1, 1.3 Hz, Ar-H), 7.53-7.68 (m, 3H, Ar-H), 8.21-8.31 (m, 2H, Ar-H), 8.47 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.6, 109.7, 111.2, 112.3, 114.2, 114.8, 116.5, 117.3, 120.2, 124.1, 124.9, 127.2, 129.3, 129.5, 131.1, 132.3, 138.2, 151.3, 157.1, 157.8 (d, *J* = 236.2 Hz), 158.2, 159.3 (d, *J* = 252.3 Hz); ^{19}F NMR ($CDCl_3$) δ: -122.3 (m, 1F), -113.1 (m. 1F); LCMS M+1: 421.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-[4-fluoro-3-(trifluoromethyl)phenyl][1,2,4]triazolo-[3,4-b][1,3,4]thiadiazole (**3b**)*

IR (KBr): 1577 (C=N), 1251 (N-N=C), 695 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.86 (s, 3H, -OCH_3), 6.91-6.94 (m, 1H, Ar-H), 7.11-7.16 (m, 2H, Ar-H), 7.25 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.41 (m, 2H, Ar-H), 7.51 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.59 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.85 (s, 1H, Ar-H), 8.32 (m, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.6, 109.2, 111.3, 113.4, 114.8, 116.1, 118.1, 121.5, 122.3, 124.2, 124.5 (q, *J* = 270.3 Hz), 125.6, 124.2, 127.5, 129.7, 130.2, 132.1, 138.8, 151.3, 157.2, 158.1 (d, *J* = 232.3 Hz), 158.2, 159.3 (d, *J* = 258.3 Hz); ^{19}F NMR ($CDCl_3$) δ: -61.1 (m, 3F), -121.2 (m, 1F), -115.1 (m. 1F); LCMS M+1: 489.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(4-iodophenyl)[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole (**3c**)*
IR (KBr): 1591 (C=N), 1265 (N-N=C), 710 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.83 (s, 3H, $-OCH_3$), 6.94-6.98 (m, 1H, Ar-H), 7.05-7.09 (m, 1H, Ar-H), 7.16 (dd, 1H, *J* = 8.1, 3.8 Hz, Ar-H), 7.61-7.71 (m, 4H, Ar-H), 7.92-7.95 (m, 2H, Ar-H), 8.38 (d, 1H, *J* = 8.1 Hz, Ar-H), 8.57 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.6, 98.9, 109.3, 110.5, 112.6, 116.3, 117.3, 120.3, 122.2, 124.5, 125.5, 127.8, 129.2, 132.3, 133.1, 139.2, 134.1, 140.3, 151.2, 157.1 (d, *J* = 234.2 Hz), 157.2, 158.1; ^{19}F NMR ($CDCl_3$) δ: -123.3 (m, 1F); LCMS M+1: 529.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-pyridin-3-yl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3d**)*
IR (KBr): 1584 (C=N), 1248 (N-N=C), 696 (C-S-C) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H, $-OCH_3$), 7.19-7.29 (m, 3H), 7.68-7.71 (m, 3H), 8.31-8.46 (m, 3H), 8.85 (d, 1H, *J* = 8.2 Hz), 9.23 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 56.6, 113.8, 114.2, 116.2, 117.1, 117.4, 124.9, 125.4, 125.6, 127.2, 128.1, 129.6, 131.6, 135.4, 141.2, 150.1, 151.1, 153.2, 155.1, 158.1 (d, *J* = 231.1 Hz), 158.5; ^{19}F NMR ($CDCl_3$) δ: -123.3 (m, 1F); LCMS M+1: 404.1.

*2-[3-(5'-fluoro-2'-methoxybiphenyl-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-1H-indole (**3e**)*
IR (KBr): 1597 (C=N), 1257 (N-N=C), 720 (C-S-C) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H, $-OCH_3$), 7.11-7.29 (m, 5H), 7.31-7.54 (m, 2H), 7.68-7.71 (m, 3H), 8.41-8.43 (m, 2H), 12.36 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 56.4, 107.2, 109.3, 110.8, 111.6, 113.2, 116.2, 120.2, 120.5, 122.1, 122.5, 123.6, 124.3, 125.2, 129.8, 131.1, 133.5, 135.2, 138.2, 138.8, 151.8, 154.5, 157.6 (d, *J* = 232.1 Hz), 157.5; ^{19}F NMR ($CDCl_3$) δ: -124.3 (m, 1F); LCMS M+1: 442.9.

*6-(2-chloro-6-fluorophenyl)-3-(5'-fluoro-2'-methoxybiphenyl-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3f**)*
IR (KBr): 1577 (C=N), 1251 (N-N=C), 697 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.81 (s, 3H, $-OCH_3$), 6.92-6.94 (m, 1H, Ar-H), 7.01-7.04 (m, 1H, Ar-H), 7.14 (dd, 1H, *J* = 9.2, 4.1 Hz, Ar-H), 7.23 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.43 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.53-7.66 (m, 3H, Ar-H), 8.35 (d, 1H, *J* = 9.1 Hz, Ar-H), 8.55 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.2, 109.4, 111.6, 113.4, 115.2, 115.9, 117.5, 118.2, 119.3, 125.2, 125.8, 127.2, 128.1, 129.1, 131.4, 135.3, 138.9, 151.2, 157.6 (d, *J* = 232.1 Hz), 157.2, 158.2, 159.3 (d, *J* = 252.1 Hz); ^{19}F NMR ($CDCl_3$) δ: -121.3 (m, 1F), -110.1 (m, 1F); LCMS M+1: 455.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(2-fluoro-5-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3g**)*
IR (KBr): 1588 (C=N), 1269 (N-N=C), 752 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.85 (s, 3H, $-OCH_3$), 6.97-7.01 (m, 1H, Ar-H), 7.05-7.11 (m, 1H, Ar-H), 7.16 (dd, 1H, *J* = 8.2, 4.1 Hz, Ar-H), 7.51-7.72 (m, 3H, Ar-H), 8.38 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.49-8.53 (m, 1H, Ar-H), 8.63 (s, 1H, Ar-H), 9.13 (dd, 1H, *J* = 8.2, 4.1 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.2, 109.5, 111.3, 112.5, 114.7, 114.9, 117.2, 117.4, 119.2, 125.2, 125.5, 127.2, 128.7, 129.1, 131.1, 138.1, 145.1, 151.1, 156.6 (d, *J* = 231.1 Hz), 157.2, 158.2, 159.5 (d, *J* = 252.1 Hz); ^{19}F NMR ($CDCl_3$) δ: -123.7 (m, 1F), -112.1 (m. 1F); LCMS M+1: 466.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(4-fluoro-3-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3h**)*
IR (KBr): 1585 (C=N), 1251 (N-N=C), 738 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.84 (s, 3H, $-OCH_3$), 6.96-6.99 (m, 1H, Ar-H), 7.05-7.11 (m, 1H, Ar-H), 7.16 (dd, 1H, *J* = 8.1, 4.1 Hz, Ar-H), 7.54-7.72 (m, 3H, Ar-H), 8.21-8.25 (m, 1H, Ar-H), 8.34-8.37 (m, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.68 (dd, 1H, *J* = 9.1, 4.1 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.2, 110.2, 112.1, 113.3, 114.9, 115.5, 117.3, 118.1, 119.3, 125.5, 128.2, 129.5, 130.2, 131.2, 131.5, 138.1, 139.9, 151.2, 157.1 (d, *J* = 231.1 Hz), 156.8, 158.2, 159.2 (d, *J* = 249.1 Hz); ^{19}F NMR ($CDCl_3$) δ: -127.1 (m, 1F), -115.1 (m. 1F); LCMS M+1: 466.

*6-(2,4-dichloro-5-fluorophenyl)-3-(5'-fluoro-2'-methoxybiphenyl-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3i**)*
IR (KBr): 1580 (C=N), 1245 (N-N=C), 785 (C-S-C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 3.84 (s, 3H, $-OCH_3$), 6.98-7.01 (m, 1H, Ar-H), 7.05-7.11 (m, 1H, Ar-H), 7.14 (dd, 1H, *J* = 8.1, 4.1 Hz, Ar-H), 7.52-8.21 (m, 3H, Ar-H), 8.25 (s, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.66 (dd, 1H, *J* = 9.1, 4.1 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 56.2, 109.8, 112.3, 114.1, 114.9, 115.7, 116.6, 118.1, 120.4, 124.3, 127.8, 128.6, 129.9, 131.1, 132.4, 133.9, 138.1, 151.2, 157.1 (d, *J* = 231.1 Hz), 156.8, 158.2, 159.2 (d, *J* = 249.1 Hz); ^{19}F NMR ($CDCl_3$) δ: -129.1 (m, 1F), -113.1 (m. 1F); LCMS M+1: 490.

*3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(5-nitrothiophen-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**3j**)*
IR (KBr): 1587 (C=N), 1252 (N-N=C), 728 (C-S-C) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H, $-OCH_3$), 7.18-7.28 (m, 3H), 7.66-7.68 (m, 2H), 8.28-8.31 (m, 1H), 8.43 (s, 1H), 8.67 (s, 1H), 8.86 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 56.6, 113.7, 113.8, 115.5, 115.8, 117.1, 117.4, 125.4, 125.6, 127.1, 129.6, 129.9, 131.6, 136.4, 138.1, 147.2, 153.1, 154.7, 156.6 (d, *J* = 231.1 Hz), 158.4; ^{19}F NMR ($CDCl_3$) δ: -129.7 (m, 1F); LCMS M-1: 452.

Biological activity

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains namely *Staphylococcus aureus, Pseudomonas aeruginosa, Eschericia coli* and *Bacillus subtilis* by cup-plate method [13]. The sterilized nutrient agar medium was distributed 100 mL each in two 250 mL conical flasks and allowed to cool to room temperature. To these media, 18-24 h grown sub-cultures were added and shaken thoroughly to ensure uniform distribution of organism's throughout the medium. Then, this agar medium was distributed in equal portions, in sterilized petridishes, ensuring that each petridish contains about 45-50 mL of the medium. The medium was then allowed for solidification. Then, cups were made with the help of a sterile cork borer (6 mm diameter) punching into the set of agar media. The solutions of required concentrations (50, 100 µg/mL) of test compounds were prepared by dissolving the compounds in DMF were filled in to the cups with 1mL of respective solution. Then, the petridishes were kept for incubation in an inverted position for 24-48 h at 37^0C, in an incubator. When growth inhibition zones were developed surrounding each cup, their diameter in mm was measured and compared with that of the standard drugs Streptomycin, Procaine penicillin.

Anticancer activity

The anticancer activity was performed against cancer cell lines HT29 (human adenocarcinoma), K293 (human kidney cancer) and MDA231 (human breast cancer) by using the MTT assay [14]. Cells were seeded at a density of 2×10^5 cells to each well in a 96 well plate and incubated for 12 h at 37^0C prior to treatment with the synthesized compounds. Cells were treated with different concentrations (10, 25, 50, 100 and 200 µg/mL) of the test compounds and incubated at 37^0C for period of 72 h. Then, 50 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added, and plates were incubated at 37^0C for 4 h. 150 µL of DMSO was added to dissolve the formazan crystals. The amount of produced purple formazan is proportional to the number of viable cells. The absorbance of each well was measured spectrophotometrically at 570 nm using ELISA micro plate reader (BIOTECH, USA). The percent inhibition of cell viability was determined with control values (without test compound) by following formula.

% inhibition = [(Control absorbance – Test absorbance) / Control absorbance] × 100. Three independent experiments in triplicate were performed for determination of sensitivity to each compound, IC_{50} were calculated and expressed in mean ±SD. 5-fluorouracil used as standard drug.

Table 1: Characterization data of compounds 2 and 3a-j.

CompoundNo.	Mol.formula	Yield (%) (microwave)	Nature	M.Pt. (^{0}C)	Analysis (%) found (calculated)		
					C	H	N
2	$C_{15}H_{13}FN_4OS$	86 -	White solid	210-212	56.91 (56.95)	4.11 (4.14)	17.69 (17.71)
3a	$C_{22}H_{14}F_2N_4OS$	65 (79)	White solid	186-188	62.81 (62.85)	3.35 (3.36)	13.35 (13.33)
3b	$C_{23}H_{13}F_5N_4OS$	61 (78)	White solid	190-192	56.51 (56.56)	2.65 (2.68)	11.44 (11.47)
3c	$C_{22}H_{14}FIN_4OS$	66 (81)	White solid	167-169	59.07 (50.01)	2.61 (2.67)	10.62 (10.60)
3d	$C_{21}H_{14}FN_5OS$	68 (77)	White solid	171-173	62.55 (62.52)	3.47 (3.50)	17.33 (17.36)
3e	$C_{24}H_{16}FN_5OS$	70 (82)	Yellow solid	181-183	65.25 (65.29)	3.69 (3.65)	15.85 (15.86)
3f	$C_{22}H_{13}ClF_2N_4OS$	60 (73)	Yellow solid	175-177	58.13 (58.09)	2.85 (2.88)	12.35 (12.32)
3g	$C_{22}H_{13}F_2N_5O_3S$	62 (72)	Off white solid	199-201	56.75 (56.77)	2.79 (2.82)	15.04 (15.05)
3h	$C_{22}H_{13}F_2N_5O_3S$	61 (81)	Yellow solid	179-182	56.75 (56.77)	2.85 (2.82)	15.08 (15.05)
3i	$C_{22}H_{12}Cl_2F_2N_4OS$	60 (77)	White solid	167-169	54.07 (54.00)	2.43 (2.47)	11.47 (11.45)
3j	$C_{20}H_{12}FN_5O_3S_2$	62 (75)	Yellow solid	208-210	52.91 (52.97)	2.61 (2.67)	15.47 (15.44)

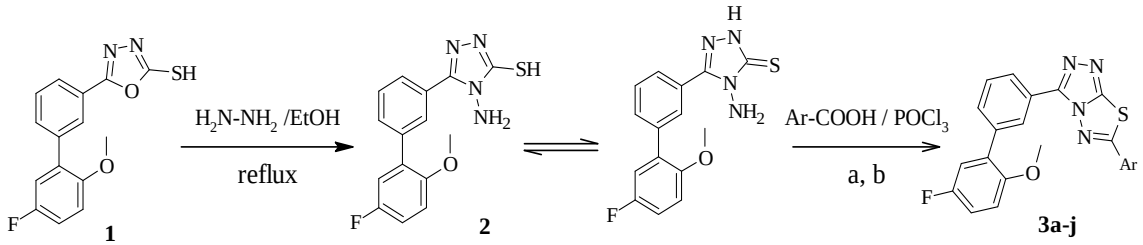
RESULTS AND DISCUSSION

Chemistry

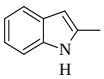
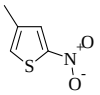
4-Amino-5-(5'-fluoro-2'-methoxybiphenyl-3-yl)-4*H*-1,2,4-triazole-3-thiol **2** was prepared in good yield by the reaction of 5-(5'-fluoro-2'-methoxybiphenyl-3-yl)-1,3,4-oxadiazole-2-thiol **1** with hydrazine hydrate according to scheme-1. Oxadiazole **1** was in turn prepared from corresponding hydrazides according to literature procedure [12].

Scheme 1: Synthesis of triazolothiadiazoles

Our initial efforts toward the synthesis of compounds **3a-j** by cyclocondensation of **2** with various substituted aromatic and heterocyclic carboxylicacids in the presence of phosphorus oxychloride involved heating at 110^0C about 7h, while under microwave irradiation it required shorter reaction time (5 minute) and higher product yields making it superior method. All the new compounds were characterized by elemental analysis, IR, ^{1}H, ^{13}C, ^{19}F NMR and mass spectral studies. The yield and physical data of synthesized compounds are summarized in Table 1.



Conditions: (a) reflux, (b) Microwave irradiation at 50°C, 70W

	Ar		Ar		Ar
3a	2-FC_6H_4	**3d**		**3g**	2-F,5-$NO_2C_6H_3$
3b	4-F,3-$CF_3C_6H_3$	**3e**		**3h**	4-F,3-$NO_2C_6H_3$
3c	4-IC_6H_4	**3f**	2-Cl,6-FC_6H_3	**3i**	2,4-Cl_2,5-FC_6H_2
				3j	

In the IR spectra of compound **2**, the disappearance of C-O-C stretching bands of **1** and appearance of $-NH_2$ bands at 3143, 3225 cm^{-1} are the evidence for conversion of **1** to **2**. In solid state the compound **2** exists in thiol form and thione form in solution. The IR spectra, recorded in KBr pellets show absorption at 2531 cm^{-1} attributed to SH peak. The predominant tautomer of **2** was identified as thione form by means of NMR spectroscopy. The NH protons observed at 13.6 ppm in the ^{1}H NMR and the carbon of C=S observed at 167.3 ppm in the ^{13}C NMR spectra is evidence for thione form of these compounds in solution. Compounds **3a-j** showed absorption peaks for N-N=C in the region of 1248-1269 cm^{-1} and for C-S-C, in the region of 690-752 cm^{-1}. The band which appeared in 1577-1597

cm^{-1} region is attributed to stretching frequency of N=C linkage of thiadiazole ring. In ^{13}C NMR of compounds **3a-j**, C-3 and C-5 of carbons triazole were observed around 137.2-138.9 ppm and 151.1-153.8 ppm respectively, while C-6 of triazolo-thiadiazole ring were seen around 155-157 ppm. In ^{19}F NMR of compounds **2** and **3a-j** the signals observed around -129.7 to -110.1 ppm was due to fluorines at the aromatic ring.

Biological activity

Antibacterial activity

The antibacterial activity results indicated that, compounds **3a-j** showed better activity than its precursor compound **2** against all the four bacterial strains. Among the compounds **3a-j**, compounds having the fluoro-substitution on the phenyl ring of thiadiazole showed better activity than the heterocyles attached to the thiadiazole ring. Compounds **3b** and **3g** showed comparatively good activity against all the bacterial strains. This is attributed due to the presence of 4-F, 3-CF_3 and 2-F, 5-NO_2 groups in the thiadiazole ring. The results are summarized in Table 2.

Anticancer activity

The anticancer activity results mentioned in Table 3 shows the cytotoxic effects of compounds **2** and **3a-j** against HT29, K293 and MDA231 cancer cell lines. Some of the compounds showed significant cytotoxic effect. 4-Amino-5-(5'-fluoro-2'-methoxybiphenyl-3-yl)-4*H*-1,2,4-triazole-3-thiol **2** showed IC_{50} value 95 µM against HT29 cell line. Construction of triazolo-thiadiazole ring increased the cytotoxicity against all the cancer cell lines. Compounds **3b** and **3g** having 4-F, 3-CF_3 and 2-F, 5-NO_2 groups in the thiadiazole ring showed significant activity against HT29 (IC_{50} 10, 13 µM), K293 (IC_{50} 20, 25 µM) and MDA231 (IC_{50} 9, 13 µM) cell lines respectively. Compounds **3f** and **3i** having 2-Cl, 6-F and 2,4-diCl, 5-F groups did not exhibit significant activity. Whereas replacement of phenyl group of the thiadiazole ring by 2-nitro thiophene (**3j**) showed moderate cytotoxic activity.

Table 2: Antibacterial activity data of the compounds 2 and 3a-j: zone of inhibition in mm

	Gram –positive bacteria				Gram –negative bacteria			
	S.aureus		*B.subtilis*		*E.coli*		*P.aeruginosa*	
Compound	50 (µg/mL)	100 (µg/mL)	50 (µg/mL)	100 (µg/mL)	50 (µg/mL)	100 (µg/mL)	50 (µg/mL)	100 (µg/mL)
2	09	11	10	12	07	13	08	12
3a	14	19	14	20	12	15	11	14
3b	16	20	16	21	18	19	16	20
3c	10	11	11	13	10	12	11	14
3d	10	13	13	14	11	12	13	11
3e	11	14	12	11	08	11	12	11
3f	10	17	11	16	10	16	13	18
3g	15	21	15	20	18	19	14	21
3h	12	15	10	17	12	19	07	15
3i	13	16	11	18	12	16	06	15
3j	10	12	13	10	11	13	09	11
Streptomycin	-	-	-	-	21	25	21	25
rocaine penicillin	22	27	24	28	-	-	-	-

Table 3:*In vitro* cytotoxicity data (IC_{50} µM) of compounds 2 and 3a-b against cancer cell lines by MTT assay

Compound No.	HT29±SD[a]	K293±SD[a]	MDA231±SD[a]
2	95±1.8	160±1.3	110±1.1
3a	83±1.3	153±1.2	94±1.8
3b	10±1.2	20±1.1	9±1.3
3c	81±1.1	145±1.2	78±1.3
3d	86±1.5	120±1.4	87±1.4
3e	75±1.7	180±1.8	90±1.5
3f	81±1.8	170±1.8	75±1.8
3g	13±1.5	25±1.1	13±1.6
3h	21±1.1	36±1.4	28±1.2
3i	52±1.3	68±1.3	75±1.1
3j	15±1.4	27±1.8	17±1.8
5-FU	8.5±1.5	41±1.1	10.1±1.1

[a]*SD = standard deviation*

CONCLUSION

In summary, we have synthesized a novel series of 3-(5'-fluoro-2'-methoxybiphenyl-3-yl)-6-(substituted)[1,2,4]-triazolo[3,4-*b*][1,3,4]-thiadiazole by conventional and microwave irradiation method. Among the two methods microwave-assisted method proved to be a rapid and better yield compare to that of conventional method. Also the

synthesized compounds were screened for their anticancer and antibacterial activity. The antibacterial and anticancer screening results showed that among the tested compounds, compound **3b** and **3g** having 4-F, 3-CF_3 and 2-F, 5-NO_2 groups in the phenyl ring of thiadiazole exhibited the highest activity.

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