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Synthesis of 3-(7-substituted-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-dimethylamino)phenyl)thiazolidin-4-ones for antibacterial, anti-inflammatory and antioxidant activity

Murugeshaiah M. B., Bhadbhade P. S.*, Rashmi P., Shachindra L. Nargund and L. V. G. Nargund

Department of Pharmaceutical Chemistry, Nargund College of Pharmacy, Dattatreya Nagar, II Main, BSK II stage, Bangalore, India

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ABSTRACT

*Fluoronitro benzothiazoles are an interesting group of compounds with a wide range of biological activities. In present work an effort has been done to find suitability and feasibility of different synthetic methods available in the literature for the preparation of fluoro-nitrobenzothiazole derivatives (or by Retro-synthetic approach). Attachment of thiazolidine in fluoro-nitrobenzothiazole ring system, has been tried in thought that it could enhance the activity of benzothiazole and other derivatives . Based on the above observations, we have synthesized some fluorobenzothiazole derivatives in hope of getting new pharmacological agents with broad spectrum of clinical activity. Compounds **9d,** and **9e** have shown moderate activity against Staphylococcus aureus. Compounds **9b,** and **9d** have shown moderate activity against **Bacillus subtillis**. The synthesized compounds **9a, 9c** and **9e** were found to possess moderate anti-inflammatory activity when compared to Diclofenec sodium. The study regarding anti oxidant activity shown that synthesised compounds are not anti-oxidants.*

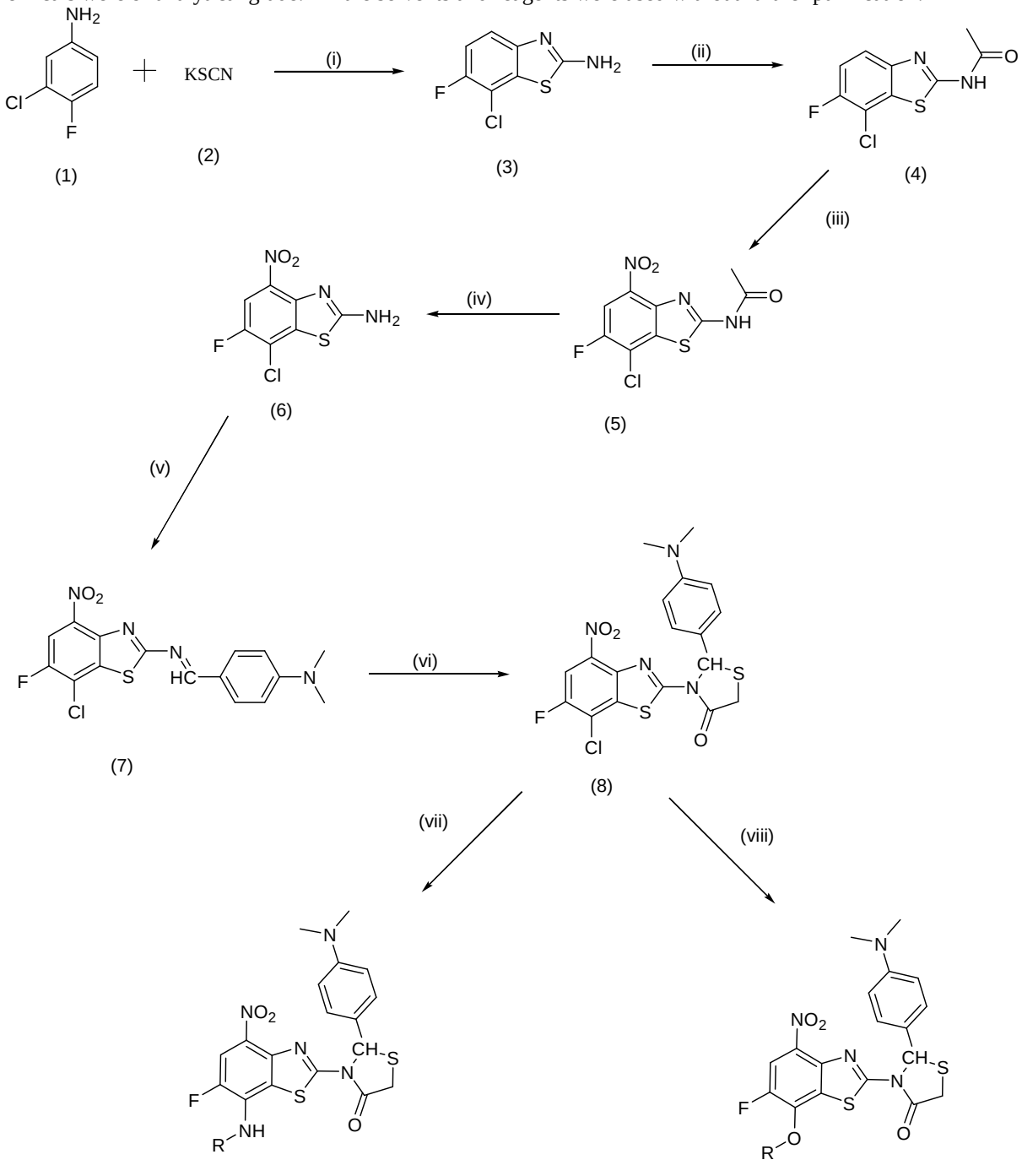
Key words: Fluorobenzothiazole, Thiazolidine, Anti-bacterial, Anti-inflammatory activity, Anti-oxidant activity.

INTRODUCTION

In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess biological activities, such as anti-tumor, anti-microbial, anthelmintic, anti-leishmanial, anti-convulsant and anti-inflammatory activity[1]. Rapid progress of organic fluorine chemistry since 1950 has been translated as a pathfinder to invent useful biodynamic agents in medicinal and biochemistry[2]. Compounds containing nitro groups (nitrophenols, nitrobenzenes and nitrofurans) possess high physiological activity. It has been shown that introduction of a nitro group into benzoylmorphine in the para position increases its analgesic activity and reduces its side effects associated with depression of the respiratory center[3]. Chloramphenicol, nitrofurans are the very good antibiotics available in the market contains nitro group in the structure. In present work an effort has been done to find suitability and feasibility of different synthetic methods available in the literature for the preparation of fluoro-nitrobenzothiazole derivatives (or by Retro-synthetic approach). Attachment of thiazolidine in fluoro-nitrobenzothiazole ring system, has been tried in the thought that it could enhance the activity of benzothiazole and other derivatives. Based on the above observations, some fluorobenzothiazole derivatives have been synthesized in the hope of getting new pharmacological agents with broad spectrum of clinical activity.

MATERIALS AND METHODS

Melting points were determined by Thiel's melting point tube(capillary tube method). The melting points were determined and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8300 Shimadzu and the frequencies were expressed in cm^{-1}. 1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard. Completion of the reaction and the purity of the compounds were checked on Merck precoated silica gel 60 F-254. Yields were not optimized. Bovine serum albumin (Merck Limited) and other chemicals were of analytical grade. All the solvents and reagents were used without further purification.



(i) Br_2, CH_3COOH, 5 °C, stir, NH_3; (ii) $(CH_3CO)_2O$, reflux 1 h; (iii) conc. HNO_3 and conc. H_2SO_4, stir, r.t. (iv) 70% H_2SO_4, reflux, 30 minute (v) N,N-Dimethyllaminobenzaldehyde, toluene in Dean-Stark apparatus, reflux 24 h (vi) Thioglycollic acid, toluene in Dean-Stark apparatus, reflux 12 h (v) Different amines, Ethanol, Triethylamine, reflux 5 h. (vi) Different phenols, Ethanol, Triethylamine, reflux 5 h.

Synthesis of 7-chloro-6-fluorobenzo[*d*]thiazol-2-amine (3)

To glacial acetic acid (100 mL) precooled at 5 °C were added 40 g (0.4123 mol) potassium thiocyanate and 7.25 g

(0.0498 mol) of 3-chloro-4-fluoro aniline. The mixture was stirred, during which 6 mL of bromine in 24 mL of glacial acetic acid was added at such a rate that the temperature should not allowed to rise beyond 5 °C, for a period of 2 h. The stirring was continued for an additional 2 h at the same temperature, and further at ro om temperature for 10 h. It was allowed to stand overnight during which an orange precipitate was settled at the bottom. 30 mL of water was added and slurry was heated at 85 °C and filtered hot. The filtrate was cooled and neutralized with ammonia, a light yellow precipitate obtained was collected. The resulting product was recrystallized by toluene[4].

Yield 76%; slight yellowish crystalline; m.p.: 180-182 °C; IR (ν, cm^{-1}, KBr): 3475 (NH_2), 1193 (C-F), 1070 (C-Cl).

Synthesis of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)acetamides (4)
A mixture of compound **1** (2.025 g, 0.01 mol) and 10 mL of acetic anhydride was refluxed for 1 h. The reaction was monitored by TLC. After completion of the reaction, mixture was cooled. Separated solid was heated with water, cool, filter and wash the residue with water. The product was then recrystallized by acetone and water[4].

Yield 98%, m.p.: 232-234 °C, whitish needle shaped crystal; IR (ν, cm^{-1}, KBr): 3318 (N-H), 1681 (C=O), 1195 (C-F), 1012 (C-Cl)

Preparation of N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)acetamides (5)
A mixture of compound **2** (100 mg, 0.000409 mol) and 0.3 mL of conc. H_2SO_4 was stirred under ice cooled condition. To this 0.1 mL conc. HNO_3 was added drop-wise and continue stirring at room temperature for 2 h. Then 0.1 mL conc. HNO_3 was further added to the reaction mixture and stirred overnight. The reaction mixture was poured into a large amount of cold water. The solid separated was filtered and washed with water thoroughly and dried under vacuum[5].

Yield 30%; mp 336-338 °C; off white crystalline solid

Deacetylation of N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)acetamides (6)
Compound **3** (1 g, 0.004040 mol), in 70% H_2SO_4 was refluxed for 30 min separately. Then poured the clear solution into 50 mL of cold water and neutralized it with sodium hydroxide solution. Filtered the solid product and recrystallized with DMF- water mixture[6].

Yield: 94%; m.p.: 192-194 °C; yellowish needle shaped crystalline solid

7-chloro-*N*-(4-(dimethylamino)benzylidene)-6-fluoro-5-nitrobenzo[*d*]thiazol-2-amine (7)
A mixture of compound **1** (0.01 mole), substituted aromatic aldehydes (0.01 mole) and 2-3 drops of glacial acetic acid in dry benzene was refluxed for 24 h in Dean-Stark apparatus.

The reaction was monitored by TLC. After completion of the reaction, mixture was kept in the refrigerator overnight; the solid product obtained was filtered and recrystallized from Toluene[7].
Yield: 73%; m.p.: 202-204 °C; yellowish crystalline solid.

Preparation of 3-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethylamino) phenyl)thiazolidin-4-one. (8)
Add Compound **8** (100mg) in Round bottomed flask containing Toluene(as solvent), Thioglycolic acid was added dropwise(2 to 3drops) in presence of Zinc chloride. Mixed well and refluxed for 10-12 hours by using deenstark apparatus. After completion, Toluene distilled off, residue diluted with ice cold water, filtered and dried the product.
Yield 50%, m.p.: 124 °C, IR (ν, cm^{-1}, KBr): 3327.32 (NH), 3253.81 (Aromatic C-H), 2958.90 (Aliphatic C-H), 1650 (C=O), 1608.69 (C-S), 1550.82 (C=C), 1448.59 (C=N), 1228.70 (C-F), 1028.70 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 6.9-8.13(m, 5H, Phenyl ring), 3.73 (s, 2H, CH_2), 3.66 (s, 2H, CH), 3.38 (s, 6H, CH_3).

Syntheses of 3-(7-Substituted-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethyl amino) phenyl)thiazolidin-4-ones (9a-9f)
Take equimolar quantity of the compound **8** (0.01 mol) and different aromatic amines(0.01 mol) or aromatic phenols and to it 5 ml of ethanol and 0.2 ml of triethylamine was added and refluxed for 5 hours. The reaction mixture was cooled and filtered. The filtered compound was dried and recrystallized using ethanol.

3-(7-(3-chloro-4-fluorophenylamino)-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethylamino)phenyl) thiazolidin-4-one
Yield 54%, m.p.: 184 °C. IR (ν, cm^{-1}, KBr): 3402 (NH), 3090.07 (Aromatic C-H), 2974.33 (Aliphatic C-H), 1650 (C=O), 1606.76 (C-S), 1550 (C=C), 1402.30 (C=N), 1249.91 (C-F), 1105 (C-Cl).

3-(7-(2-chlorophenylamino)-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethylamino) phenyl)thiazolidin-4-one.
Yield 58%, m.p.: 204 °C. IR (ν, cm^{-1}, KBr): 3292 (NH), 3090.07 (Aromatic C-H), 2972.40 (Aliphatic C-H), 1650 (C=O), 1639 (C-S), 1546.96 (C=C), 1402.30 (C=N), 1193.98 (C-F), 1070.53 (C-Cl).

3-(7-(3-chlorophenylamino)-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)- 2-(4-(dimethyl amino)phenyl)thiazolidin-4-one
Yield 58%, m.p.: 206 °C. IR (ν, cm^{-1}, KBr): 3306 (NH), 3090.07 (Aromatic C-H), 2968.55 (Aliphatic C-H), 1701 (C=O), 1639.55 (C-S), 1587.47 (C=C), 1402.30 (C=N), 1261.49 (C-F), 1068.60 (C-Cl).

2-(4-(dimethylamino)phenyl)-3-(6-fluoro-7-morpholino-4-nitrobenzo[d]thiazol-2-yl) thiazolidin-4-one
Yield 55%, m.p.: 214 °C. IR (ν, cm^{-1}, KBr): 3306 (NH), 3099.71 (Aromatic C-H), 2960.83 (Aliphatic C-H), 1701 (C=O), 1606.76 (C-S), 1548.89 (C=C), 1442.80 (C=N), 1247.99 (C-F), 1053.17 (C-Cl).

2-(4-(dimethylamino)phenyl)-3-(6-fluoro-4-nitro-7-(*o*-tolyloxy)benzo[d]thiazol-2-yl) thiazolidin-4-one
Yield 55%, m.p.: 164 °C. IR (ν, cm^{-1}, KBr): 3306 (NH), 3099.71 (Aromatic C-H), 2960.83 (Aliphatic C-H), 1701 (C=O), 1606.76 (C-S), 1548.89 (C=C), 1442.80 (C=N), 1247.99 (C-F), 1053.17 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 6.821-7.560 (m, 5H, Phenyl ring), 3.73 (s, 2H, CH_2), 3.66 (s, 2H, CH), 2.925 (s, 3H,CH_3), 1.635 (s, 6H, CH_3).

***In vitro* anti-microbial activity**
Anti-microbial activity is determined based on the ***in-vitro*** activity against ***Staphylococcus aureus*** (Gram +ve) and ***Bacillus subtillis*** (Gram –ve). The media were cooled to room temperature and inoculated with test organism (20 ml of subculture medium/100 ml assay medium) 30 ml aliquots of inoculated media were distributed into each of petri plates and maintained at room temperature to solidify. The cups (8 mm) were bored using cork borer. The test solution and the standard drugs in two different concentrations viz., (50 mcg/ml and 10 mcg/ml) were placed in the so made cups. The volume of the test and standard solutions added was 0.1 ml using sterile pipettes. All the above operations were carried out in an aseptic area under laminar flow. The petri plates were kept in the refrigerator for 2 hours to allow uniform diffusion of the drug into the agar medium. Later they were taken out from the refrigerator and incubated for 24-36 hours at 37+/-1 °C. After the incubation period was over, the plates were observed for zone of inhibition and were measured using transparent scale or slide calipers, each reading was taken in triplet. The average mean zone of inhibition was listed in the table[10-12].

***In vitro* anti-inflammatory activity**
The test compounds were dissolved in minimum amount of Dimethyl formamide (DMF) and diluted with Phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was 2% test solution (1ml) containing different concentrations of drug were mixed with 1 ml of 1% mM bovine serum albumin in phosphate buffer and incubated at 270 °C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60 °C in a water bath for 10 min[13]. After cooling, the turbidity was measured at 660nm (Shimadzu UV visible spectrometer). Percentage of denaturation was calculated from the following formula:

% Inhibition = 100(1-Vt/Vc)

Where Vt = absorbance value in test solution.
Vc = absorbance value in control solution.

Antioxidant activity
The reaction mixture containing *o*-phenanthroline (0.5m), ferric chloride (0.2mM) and different type fractions of test compound in a volume of 5 ml was incubated for 15-20 min at ambient temperature. The absorbance at 510nm was measured. In other set, sodium dithionite (0.3mM) was added instead of test samples and the absorbance was taken as equivalent to 100% reduction of all the ferric ions present[14].

Anti-oxidant activity can be calculated by the following formula:

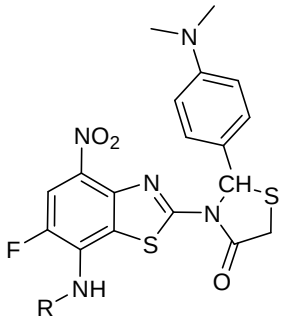
% activity = [At /As] × 100

Where, At = Absorbance by Sodium dithionite (300 µg/ml) at 510 nm.
As = absorbance by standard drug solution at 510 nm.

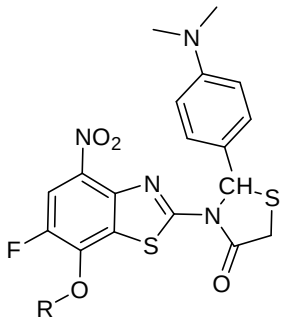
RESULTS AND DISCUSSION

In present work for the preparation of 2-amino-7-chloro-6-fluorobenzothiazole, 4-Fluoro-3-chloroaniline was allowed to react with Potassium thiocyanate in Glacial acetic acid, in the presence of bromine, to form 2-Amino-7-chloro-6-fluorobenzothiazole. It was further treated with Acetic anhydride and refluxed gently under short air condenser for 10-15 Min. Cooled the reaction mixture and poured it in to 200 ml of cold water and boiled to decompose the excess of acetic anhydride. Filtered the residue and washed with little cold water. The product was recrystallised from acetone and water. Further a series of reactions were done and the product 7-chloro-*N*-(4-(dimethylamino)benzylidene)-6-fluoro-5-nitrobenzo[*d*]thiazol-2- amine (7) was treated with Thioglycolic acid in presence of Zinc chloride in toluene to get 3-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethylamino)phenyl) thiazolidin-4-one(8). The obtained compound (8) is treated with different amines and phenols to get different derivatives. All the compounds synthesized were identified and characterized by physical methods like Melting point (**Table 1**), thin layer chromatography and spectral methods like IR and ^{1}HNMR spectra.

Table-1: Physicochemical properties of the synthesized compounds 9(a-f), 10(a-d)



(9a-9f)



(10a-10d)

Comp.	R	m.p.(°C)	% Yield	R_f value
9a	C_6H_4-4-F-3-Cl	185	54%	0.34
9b	C_6H_4-2- Cl	205	58%	0.22
9c	C_6H_4-3- Cl	168	58%	0.18
9d	C_6H_4-4- Cl	200	50%	0.20
9e	N-yl-Morpholine	215	55%	0.24
9f	N-yl-Piperazine	185	48%	0.40
10a	C_6H_4-2-CH_3	165	55%	0.54
10b	C_6H_4-3-CH_3	205	53%	0.50
10c	C_6H_4-4-CH_3	210	48%	0.40
10d	C_6H_5	175	49%	0.40

TLC solvent: Petrileum ether, Ethylacetate: 1:1

The newly synthesized compounds were screened for anti-microbial activities against *Staphylococcus aureus* (gram +ve) and *Bacillus subtillis* (gram –ve). All the compounds were found to possess weak to moderate anti-bacterial activity against both micro-organisms when compared to Ampicillin (gram +ve) and Streptomycin (gram –ve) (**Table 2**). Compounds **9d,** and **9e** have shown moderate activity against *Staphylococcus aureus.* Compounds **9b,** and **9d** have shown moderate activity against ***Bacillus subtillis.*** The synthesized compounds were subjected to *in vitro* anti-inflammatory activity using bovine serum albumin denaturation model. **9a, 9c** and **9e** were found to possess moderate anti-inflammatory activity when compared to Diclofenec sodium (**Table 3**).

Among the synthesized two compounds **9b** and **9d** were subjected for the evaluation of anti-oxidant activity. Both the compounds were found to possess weak anti-oxidant activity (**Table 4**).

Table-2: Antibacterial evaluation data of the synthesized compounds

Compound code	ZONE OF INHIBITION AFTER 24 Hrs. (in mm) &(ACTIVITY INDEX)			
	S.aureus		*Bacillus subtilis*	
	50 mcg	100 mcg	50 mcg	100 mcg
9a	3.6mm	4.8mm	2.4mm	3.2mm
9b	3.5mm	5.5mm	5.0mm	6.2mm
9c	3.8mm	6.0mm	3.4mm	4.9mm
9d	4.2mm	4.6mm	3.6mm	5.4mm
9e	4.4mm	4.3mm	3.8mm	4.8mm
Control	4 mm	3.6 mm	1.6mm	2.3mm
Ampicillin	8.7 mm	9.4mm		
Streptomycin	-------	-------	19	28

Table 3: Anti inflammatory activity of the synthesised compounds

Sl. No	Compound Code	Absorbance value	Inhibition of Denaturation (%)
2	9a	**0.028**	**56.00**
3	9b	0.032	4.00
4	9c	**0.058**	**62.50**
5	9d	0.068	32.00
6	9e	**0.054**	**52.50**
7	Control	**0.05**	
8	**Diclofenec Sodium**	**0.08**	**97.50**

Table 4: Anti oxidant activity of the synthesised compounds

Sr. No.	Sample Code with Concentration (µg/ml)	Absorbance At 510 nm (As)	Anti-Oxidant Activity (%)
01.	$9b_{100}$	0.026	4.00%
02.	$9b_{200}$	0.017	2.60%
03.	$9b_{300}$	0.023	3.5%
04.	$9b_{400}$	0.021	3.00 %
05.	$9b_{500}$	0.022	3.3%
06.	$9d_{100}$	0.017	2.6%
07.	$9d_{200}$	0.018	2.70%
08.	$9d_{300}$	0.019	2.9%
09.	$9d_{400}$	0.027	4.1%
10.	$9d_{500}$	0.035	5.30%
11.	Std. Sodium dithionate	0.646	

CONCLUSION

A series of targeted compounds [3-(7-Substituted-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-2-(4-(dimethyl amino) phenyl)thiazolidin-4-ones] were synthesized by the two schemes and all derivatives were identified and characterized. Compounds 9d, and 9e have shown moderate activity against *Staphylococcus aureus.* Compounds 9b, and 9d have shown moderate activity against *Bacillus subtillis.* The synthesized compounds 9a, 9c and 9e were found to possess moderate anti-inflammatory activity when compared to Diclofenec sodium. The study regarding anti oxidant activity shown that synthesised compounds are not anti-oxidants.

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