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Synthesis and antimicrobial evaluation of novel ethyl 2-(2-(4-substituted) acetamido)-4-subtituted-thiazole-5-carboxylate derivatives

Chandrakant D. Pawar,[a*] Aniket P. Sarkate,[a] Kshipra S. Karnik,[a] Sushilkumar S. Bahekar,[b] Dattatraya N. Pansare,[a] Rohini N. Shelke,[c] Chetan S. Jawale,[d] Devanand B. Shinde[e]

[a]*Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431004 (MS), India*

[b]*Bond Chemicals, Aurangabad, 431004 (MS), India*

[c]*Department of Chemistry, Deogiri college, Station road, Aurangabad 431 005, MS, India.*

[d]*H.P.T.Arts & R.Y.K.Science College, College Road, Nashik-05 (MS), India*

[e]*Shivaji University,Vidyanagar, Kolhapur- 416 004(MS), India*

**Corresponding author E-mail: dbschandrakant15@rediffmail.com*

Tel No.: +91 0240-2403308 Fax No.: +91 0240-2400413

Abstract

A series of novel molecules containing thiazole ring structure were designed and synthesized. The structures of the synthesized compounds were elucidated and confirmed by ^{1}H NMR, ^{13}C NMR, Mass spectrum and the purity was checked through HPLC analysis. Among these synthesized compounds, **3a-3i** and **6a-6c** were tested for their antimicrobial activity (minimum inhibitory concentration) against a series of strains of *Bacillus subtilis, Staphyllococcus aureus* and *Esherichia coli* for antibacterial activity and against the strains of *Candida albicans, Aspergillus flavus* and *Aspergillus nigar* for antifungal activity respectively. The results of the antimicrobial screening data revealed that most of the tested compounds showed moderate to good microbial inhibitions.

Keywords: Thiazole ring, Antibacterial activity, Antifungal activity.

The thiazole nucleus is an important component for a huge spectrum of therapeutic agents including anticancer, anticonvulsants, antifungal and antibacterial agents.[1] This structure has found applications in drug development for the treatment of cardiotonic, fungicidal, HIV infection, mental retardation in children, age related and neurodegenerative brain damage (Alzheimer's disease, Parkinson's disease).[2] This class of heterocyclic compounds are found in many potent biologically active molecules such as Sulfathiazol (antimicrobial drug), Ritonavir (antiretroviral drug), Abafungin (antifungal drug) with trade name Abasol cream and Bleomycine and Tiazofurin (antineoplastic drug).[3]

Furthermore, some thiazoles are used in agricultureas pesticides and plant growth regulators. Several novel thiazole derivatives have been reported in literatures such as, introduction of fluorine into thiazoline and synthesis of sydnonyl substituted thiazolidinone and thiazoline derivatives.[4] Thiazole ring is an important pharmacophore and its coupling with other ringscould furnish new biologically active compounds.[5]

In recent times, the applications of thiazoles were found in drug development for the treatment of allergies, hypertension, inflammation, schizophrenia, bacterial, HIV infections, and hypnotics and more recently for the treatment of pain, as fibrinogen receptor antagonists with antithrombotic activityand as new inhibitors of bacterial DNA gyrase B.[3]

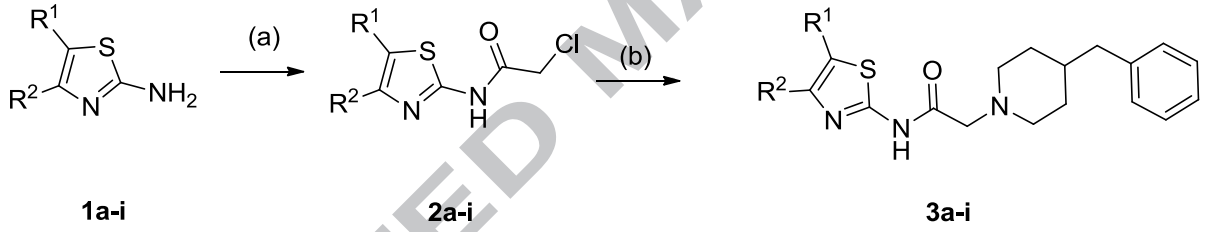
Thiazole moiety has been already reported for its antimicrobial activity. Thiazole ring is an important pharmacophore and their couplings with other ringscould furnish new biologically active compounds. Thiazole containing compounds exhibit a wide range of biological properties,such as antitumor, anticonvulsant,[6] cardiotonic,[7] IMP dehydrogenase inhibiton,[8] analgesic,[9] anticancer.[10] It was observed that, benzotriazole and thiazole rings present in the same molecule could be convenient models for investigation of their biological activity.[5] Literature revealed that syntheses of such thiazolyl-benzotriazole showed anti-convulsant and anti-inflammatory activity,[11] anti-tumoral activity.[12] After extensive literature search, it was observed that, thiazole coupling with a piperidine moiety would increase the chances of potent antimicrobial activity of the thiazole containing compound.[13-19] We herein report the synthesis of new substituted thiazole derivatives (Scheme 1 and Scheme 2) with the aim of investigating their antimicrobial activity.

Thiazoles show many biological activities so we planned to study its antimicrobial activity along with the incorporation of substituted piperidones, as substituted piperidones shows

variety of biological activities. The piperidine moiety is a very important pharmacophore because of its presence in numerous alkaloids, pharmaceuticals and diverse applications in medicinal chemistry.[20] The nucleus, as like piperidine and its derivatives are reported in literature for varied pharmacological activities like antifungal,[21] antibacterial,[22] AChE inhibitors,[23] antitubercular,[24] antihistaminics,[25] antitumor agents[26] and anticancer activity.[27] The piperidine and its derivatives are important building blocks in the synthesis of pharmaceutical drug molecules like paroxetin, methylphenidate, raloxifene, minoxidil, risperidone and pethidine. The biologically active alkaloids containing substituted piperidine ring systems have been targeted by medicinal chemists, for their complete or partial synthesis.[28]

Our research group previously reported synthesis, characterization and antimicrobial evaluation of derivatives of thiazole and thiazolidinone.[29] Here, we wish to mention the development and incorporation of thiazole and substituted piperidine scaffold in one framework.

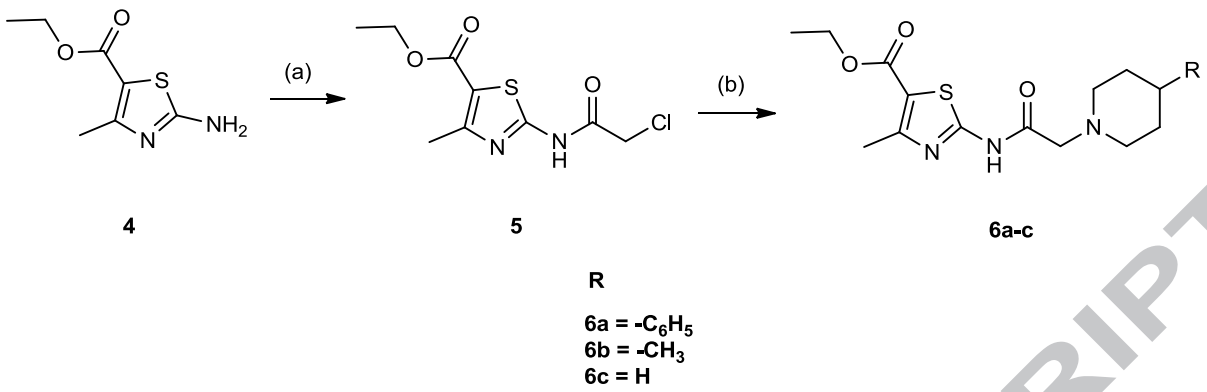
The synthetic methods adopted for the preparation of the title compounds **3a-3i** and **6a-6c** care depicted in the schemes presented below.



		R^1	R^2
1a =	2a =	$-OOC-C_2H_5$	$-CH_3$
1b =	2b =	$-OOC-C_2H_5$	H
1c =	2c =	$-OOC-C_2H_5$	$-C_2H_5$
1d =	2d =	$-OOC-CH_3$	H
1e =	2e =	$-OOC-CH_3$	
1f =	2f =	$-OOC-C_2H_5$	$-C_6H_5$
1g =	2g =	$-OC-CH_3$	$-CH_3$
1h =	2h =	$-CH_3$	$-CH_3$
1i =	2i =	H	$-CH_3$

Scheme 1 Synthesis of ethyl 2-(2-(4-Substituted) acetamido)-4-subtituted-thiazole-5-carboxylate derivatives **(3a-3i)**

Reagents and conditions: (a) 2,6-leutidine, DMAP, DCM, Chloroacetyl chloride at 0°C; (b) 2,6-leutidine, DMAP added in 4-benzylpiperidine solution in THF.



Scheme 2 Synthesis of ethyl 2-(2-(4-Substituted) acetamido)-4-subtituted-thiazole-5-carboxylate derivatives

Reagents and conditions: (a) 2,6-leutidine., DMAP, DCM , Chloroacetyl chloride at 0°C; (b) 2,6-leutidine, DMAP, 4-phenylpiperidine (2a) / 4-methylpiperidine (2b) / piperidine (2c) solution in THF.

Table 1 Screening of base and solvent for synthesis of compounds **2a-2i** and **5** step **(a)**

Entry	Base	Solvent	Time (h)	Yield[a] (%)
1	TEA (2 eq)	DMF	6	45
2	DIPEA(2 eq)	DMF	16	30
3	DMAP (2 eq)	DMF	16	55
4	2,6-Leutidine (2 eq)	DMF	6	58
5	Pyridine (2 eq)	DMF	16	40
6	DMAP (2 eq)	DCM	16	60
7	2,6-Leutidine (2 eq) and DMAP (0.2 eq)	DCM	3	80
8	DIPEA(2 eq)	DCM	16	40
9	TEA (2 eq) and DMAP (0.2 eq)	DCM	3	40
10	Pyridine (2 eq)	DCM	16	30

[a] Isolated yield.

We have optimized condition for the preparation of our substituted products by varying different bases, varying solvents and reaction time. We presented optimization conditions for both the schemes of step (a) and (b) in Tables 1 and Tables 2 respectively. For the step (a), In the base TEA with DMF solvent gives the corresponding product in a 45% yield, which was the worst among these solvents (Table 1, entry 1). Nevertheless, all of these yields were generally low before further optimizations. To increase the efficiency of the condensation reaction, the effects of different solvents were investigated (Table 1, entries 1-10). DCM exhibited the best performance of the solvent; the product yield was 80%, reaction completion time only 3 h (Table 1, entries 7). The DMF gave lower yields as a solvent with used bases (Table 1, entries 1-3, and 5). But interestingly in the base 2,6-Leutidine the product yield was 58%, All the reactions were carried out with various amounts of each bases in 1 mL of solvent. Among these reactions same amounts of the solvent, 1 mL of DCM turned out to be the best choice with 2,6-Leutidine (2 eq) and DMAP (0.2 eq) gives yield of 80% (Table 1, entry 7). We like to mention here in DCM as a solvent and with 2,6-Leutidine (2 eq) and DMAP (0.2 eq) were the best choice and less time required for reaction completion. We decided to carry out the further reaction in DCM.

Table 2 Screening of base and solvent for synthesis of compounds **3a-3i** and **6a-6c** step **(b)**

Entry	**Base**	**Solvent**	**Time (h)**	**Yield[a] (%)**
1	2,6-Leutidine (2 eq) and DMAP (0.2 eq)	THF	6	90
2	2,6-Leutidine (2 eq) and DMAP (0.2 eq)	DMF	16	70
3	2,6-Leutidine (2 eq) and DMAP (0.2 eq)	DCM	16	30
4	2,6-Leutidine (2 eq) and DMAP (0.2 eq)	Acetone	16	40
5	Pyridine (2 eq)	DMF	16	30
6	DMAP (2 eq)	DCM	16	40
7	DIPEA(2 eq)	DCM	16	40
8	TEA (2 eq) and DMAP (0.2 eq)	DCM	12	40

[a] Isolated yield.

For the step (b), we have again used different bases and solvents and finally optimized one condition that gives good yield along with reduced reaction time (Table 2, entry 1) without purification and crude used further for next reactions.

It was observed from the that 2,6- leutidine (2eq) and DMAP (0.2eq) as a base along with THF as the solvent gave the highest yield of 90% and the time required for the reaction was also reduced to 6 h (Table 2, entry 1), hence it was chosen as the base and the solvent for the synthesis of step (b). Hence we decided to carry out the further reaction in THF as a solvent and 2,6- leutidine (2eq), DMAP (0.2eq) as a base. All synthesized compounds of ethyl 2-(2-(4-substituted) acetamido)-4-subtituted-thiazole-5-carboxylate derivatives (**3a-3i** and **6a-6c**)[30] are shown in Table 3.

All the synthesized compounds were screened for *in vitro* antimicrobial activity. The antibacterial activity was evaluated against different bacterial strains such as *Staphylococcus aureus* (NCIM-2901), *Bacillus subtilus* (NCIM-2063) and *Escherichia coli* (NCIM-2256). Minimum inhibitory concentration (MIC, µg/mL) of antibacterial activity was determined using broth dilution methodas per CLSI guidelines.[31-36] Levofloxacin was used as a standard drug for

Table 3 Synthesis of ethyl 2-(2-(4-Substituted) acetamido)-4-subtituted-thiazole-5-Carboxylate Derivatives

Compound	**Reactant (Step a)**	**Reactant (Step b)**	**Melting point (°C)**	**Yield (%)**
3a	Ethyl-2-amino-4-methylthiazole-5-carboxylate	4-benzylpiperidine	143	82
3b	Ethyl-2-aminothiazole-5-carboxylate	4-benzylpiperidine	118	90
3c	Ethyl-2-amino-4 ethylthiazole-5-carboxylate	4-benzylpiperidine	153	90
3d	Methyl-2-aminomethylthiazole-5-carboxylate	4-benzylpiperidine	117	90
3e	Methyl-2-amino-4-	4-benzylpiperidine	113	90

	cyclopropylthiazole-5-carboxylate			
3f	Ethyl-2-amino-4-phenylthiazole-5-carboxylate	4-benzylpiperidine	153	89
3g	1-(2-amino-4-methylthiazol-5-yl)ethanone	4-benzylpiperidine	127	88
3h	4,5-dimethylthiazol-2-amine	4-benzylpiperidine	118	87
3i	4-methylthiazol-2-amine	4-benzylpiperidine	108	92
6a	Ethyl-2-amino-4-methylthiazole-5-carboxylate	4-phenylpiperidine	173	92
6b	Ethyl-2-amino-4-methylthiazole-5-carboxylate	4-methylpiperidine	147	90
6c	Ethyl-2-amino-4-methylthiazole-5-carboxylate	piperidine	133	86

Table 4.Antimicrobial activity of the synthesized compounds (**3a-3i and 6a-6c**)

Compound	**MIC values[a] (µg/ml)**					
	B. subtilis	***E. coli***	***S. aureus***	***C. Albicans***	***A. Flavus***	***A. Niger***
3a	35	50	70	75	50	50
3b	**29**	**29**	**28**	**25**	**12.5**	**12.5**
3c	40	100	100	75	100	100
3d	35	60	50	100	50	50
3e	**29**	**29**	**28**	**25**	**12.5**	**12.5**
3f	**25**	**29**	**28**	**50**	**25**	**25**
3g	35	60	50	100	50	50
3h	**28**	**29**	**30**	**25**	**12.5**	**12.5**
3i	35	50	70	75	50	50
6a	**28**	**29**	**30**	**25**	**12.5**	**25**
6b	35	50	70	75	50	50
6c	45	60	100	75	100	100

Levofloxacin	**29**	**29**	**28**	**-**	**-**	**-**
Fluconazole	**-**	**-**	**-**	**40**	**25**	**25**
Miconazole	**-**	**-**	**-**	**12.5**	**12.5**	**12.5**

[a]Values are the average of three readings.

the comparison of antibacterial activity (Table 4). Fluconazole and miconazole were used as standard drugs for the comparison of antifungal activity. Dimethyl sulfoxide was used as solvent control. From the antimicrobial data, it is observed that all the newly synthesized compounds shows good to moderate level of antibacterial and antifungal activity (Table 4). The antimicrobial activity data reveals that compounds (**3b, 3e, 3f, 3h** and **6a**), are found to be most active and potent as antimicrobial agents among the series.

The antimicrobial activity data reveals that among the synthesized compounds **3b, 3e, 3f, 3h** and **6a** are found to be most active and potent antimicrobial agents among the series when compared with the standard. The compound **3c** as well as **6c** containing the ethyl and methyl moiety on the thiazole ring and no substitution on the piperidine ring showed reduced antimicrobial activity. The compounds **3a, 3d and 3g** containing methyl as well as hydrogen moiety on the thiazole ring showed intermediate antibacterial activity. The same compounds **3b, 3e, 3f, 3h** and **6a** showed higher antifungal activity when tested on the fungal strains. The structure-activity relationship of the series can be explained as follows:

- *Effect of alkyl group substitution on the thiazole ring (R^2)*: The molecules gave increased antimicrobial activity due to the presence of methyl as well as hydrogen group on thiazole ring. The activity was further found to be increased when there was the presence of more bulky groups on the ring like cyclopropyl and phenyl.
- *Effect of ester group substitution on the thiazole ring (R^1)*: The activity was found to be increased from good to potent when ethyl ester group was replaced by methyl ester on the ring.
- *Effect of substitution on the piperidine ring:* The activity was observed only when there was the presence of bulky groups on the piperidne ring like benzyl and phenyl. The activity decreased when less bulky groups were substituted on the ring.

In conclusion by using this methodology, substituted thiazole-5-carboxylate derivatives were synthesized in gram scale. All the compounds are purified through column chromatography by using different proportions and ethyl acetate and hexane. We have developed simple and continent method for the synthesis of some novel substituted thiazole derivatives through a reaction of substituted thiazole carboxylates and piperidines by simple reaction steps. No costly reagents are required, no any pre-purification is needed and all the compounds synthesized were obtained in good yields. We developed methodologies for the synthesis of substituted thiazoles on a laboratory scale. The mild reaction conditions, shorter reaction time and promising antimicrobial activity of the compounds (**3b, 3e, 3f, 3h** and **6a**) compared to the standard are the advantage of the present method.

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30. *General experimental procedure for synthesis of compound 3a-3i:*

Step (a): To a stirred solution of Amino-methylthiazole-5-carboxylate (1 eq) in DCM (10 times) was added 2,6-Leutidine (2 eq) and DMAP (0.2 eq) the mixture was stirred at rt for 15 min. RM was cooled to 0°C and Chloroacetyl chloride CAC (1.2 eq) was added drop wise followed by stirring at rt for 3 h. The reaction was monitored by TLC and LCMS, after completion of reaction evaporated reaction mass under reduced pressure to obtain crude gummy material. The RM was diluted with water (10 times of reaction) and extracted with DCM twice. The organic layer was washed with saturated sodium bicarbonate solution, and brine solution, organic layer was evaporated under reduced pressure to get desired product. The product was confirmed by LCMS and used as such for next step.

Step (b): To a stirred solution of substituted 4-benzyl piperidine (0.4 g, 2.29 mmol) in dry THF (10 ml) was added 2,6-leutidine (0.4 g, 3.81 mmol) and DMAP (0.046 g, 0.38 mmol) at room temperature and then the above obtained product was sequentially added to the formed reaction mixture (0.5 g, 1.90 mmol). The reaction mixture was stirred at rt for 6 h TLC shows starting material was consumed. The evaporated reaction mass was reduced under pressure to obtain crude compound as gummy material. This was washed with 20 % EtoAc:Hexane (20 ml) and 20% Acetone:Hexane (20 ml) to obtain solid compounds which are finally washed with 100% diethyl ether (10 ml)and 100 % pentane (10 ml) to obtain crude compound as solids. Purification of crude compound done by silica gel (100-200 mesh) column chromatography by using 50% EtoAc: Hexane to obtain white solid compound.

General experimental procedures for synthesis of compound 6a-6c:

Step (a): To a stirred solution of Ethyl 2-amino-4-methylthiazole-5-carboxylate (1 eq) in DCM (10 times) was added 2,6-Leutidine (2 eq) and DMAP (0.2 eq) the mixture was stirred at rt for 15 min. RM was cooled to 0°C and Chloroacetyl chloride CAC (1.2 eq) was

added drop wise followed by stirring at rt for 3 h.Reaction was monitored by TLC and LCMS , after completion of reaction evaporated reaction mass under reduced pressure to obtain crude gummy material. RM was diluted with water (10 times of reaction) and extracted with DCM twice. Washed organic layer with saturated sodium bicarbonate solution, and brine solution, organic layer evaporated under reduced pressure to get desired product. The product was confirmed by LCMS and used as such for next step.

Step (b): To a stirred solution of 4-phenylpiperidine/ 4-methylpiperidine/ piperidine (1.2eq) in dry THF (80 mL) was added 2,6-leutidine (2 eq) and DMAP (0.2 eq) at room temperature and then the above obtained product was sequentially added to the formed reaction mixture (1 eq). The reaction mixture was stirred at rt for 6 h. TLC shows starting material was consumed. The evaporated reaction mass was reduced under pressure to obtain crude compound as gummy material. This was washed with 20 % EtoAc:Hexane and 20% Acetone:Hexane to obtain solid compounds which are finally washed with 100% diethyl ether and 100 % pentane to obtain crude compounds as solids. All obtained compounds are purified by column chromatography by using 30-70: EtoAc-Hexane to obtain all compounds as white solids.

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Graphical Abstract

Synthesis and antimicrobial evaluation of novel ethyl 2-(2-(4-substituted) acetamido)-4-subtituted-thiazole-5-carboxylate derivatives

Chandrakant D. Pawar, Aniket P. Sarkate, Kshipra S. Karnik, Sushilkumar S. Bahekar, Dattatraya N. Pansare, Rohini N. Shelke, Chetan S. Jawale, Devanand B. Shinde