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Synthesis, characterization and antimicrobial activities of amide derivatives of febuxostat intermediate

Sreedhar Badvel[1], Veera Reddy Tummaluru[1], Venugopal Reddy Katta[1], Vidya Sagar Reddy Gandi[2], Naga Raju Chamarthi[*3] and Padmavathi Venkatapuram[3]

[1]*Department of Chemistry, Vikrama Simhapuri University, Nellore-524 003, India.*

[2]*Department of Biotechnology, Vikrama Simhapuri University, Nellore-524 003, India.*

[3]*Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, India.*

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Abstract: A series of new carboxamide derivatives of 3-(4-(5-(ethoxycarbonyl)-4-methylthiazol-2-yl)phenoxy)propionic acid were synthesized by Schotten-Baumann reaction. The newly synthesized compounds were characterized by IR, NMR and mass spectral analysis. The target molecules were evaluated for their efficacy as antimicrobial agents *in vitro* by disc diffusion method. Compounds **4c, 4f, 4g** and **4i** showed high inhibitory activity.

Keywords: Febuxostat intermediate; carboxamide derivatives; antimicrobial activity.

1. Introduction

Gout disease has been increasing in recent decades, due to increasing risk factors like metabolic syndrome, longer life expectancy and changes in diet. By lowering uric acid levels can cure the disease.[1] XOI (Xanthine Oxidase Inhibitors) which inhibit the XO (Xanthine Oxidase), this catalyzes hypoxanthine to uric acid.[2] Xanthine oxidase inhibitors include purine analgues Allopurinol,[3,4] Oxypurinol, Probenecid, Pterin and 6-formylpterin[5] (pteridine analogues) and non-purine analogue febuxostat are used as xanthine oxidase inhibitors. It was reported recently that the selenium containing heterocycles act as potent Xanthine oxidase inhibitors.[6]

The literature reveals that the inhibition of XO augmented by core moieties containing heteroaryl, naphthyl and phenyl fragments.[7,8] The thriving demand for introducing amide functionality in compounds of pharmaceutical, chemical and natural sectors[9-11] has grabbed intensive attention from all over the world. Besides, it is well known that a number of nitrogen, sulfur containing heterocyclic compounds exhibited a wide variety of biological activities. Various drugs like Ketoconazole, Ciprofloxacin, available in the market have Piperazine in their structure and showed potent antimicrobial activity. Piperazine derivatives were used as antianginals,[12] analgesics,[13] and antidepressants[14] and also in veterinary medicines which combat parasitic infections in poultry, stimulants at low doses, and hallucinations at higher level doses.[15]

[*]Corresponding author: E-Mail: rajuchamarthi10@gmail.com; Ph: +91 9703193375

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Benzothiazole nucleus has its own biological importance in medicinal chemistry. Riluzole, Pramipexole are examples of the drugs containing this moiety. Benzothiazole derivatives were used as potential anti-tumor,[16] anti-Alzheimer's agents,[17-19] Kv1.3 ion channel blockers,[20] analgesic, anti-inflammatory[21] and Raf-1 inhibitor.[22] Based on these findings, we have designed and synthesized a series of febuxostat derivatives with bio-potent amines and evaluated their anti-microbial activities *in vitro*.

2. Results and discussion

The synthetic pathway to the targeted compounds **4(a-j)** is sketched in **Scheme 1**. In brief, commercially available Febuxostat (**1**), 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester which was reported[23] as starting material. It was converted into acid (**2**) by the reaction of **1** with sodium hydride followed by 3-bromo propionic acid in THF as solvent. Acid chloride was synthesized by reacting the acid (**2**) with thionyl chloride, which in turn reacted with various bio-active amines described in **Scheme 1** in the presence of acid scavenger, N-methylimidazole[24-27] as a base in THF as solvent at 10-30 °C.

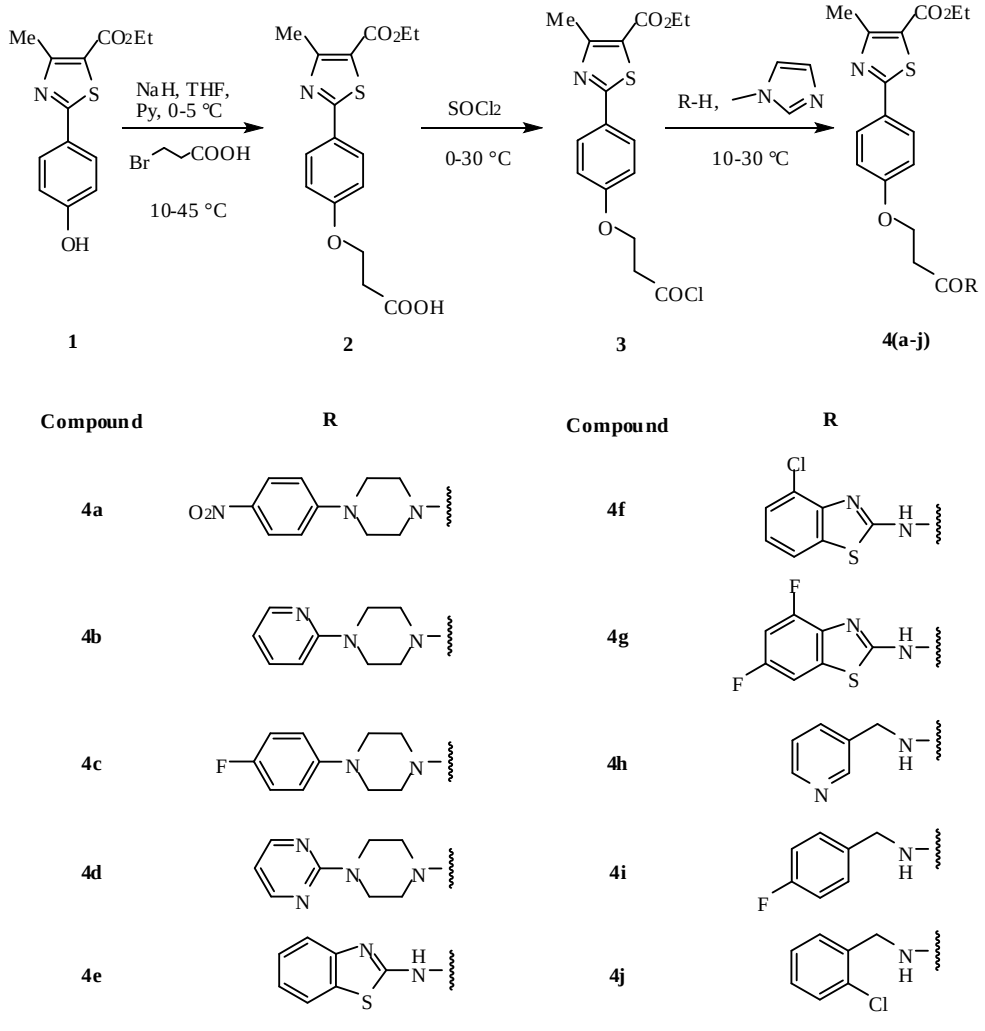

Figure 1. Synthesis of carboxamide derivatives **4(a-j)** of Febuxostat Intermediate

The reaction of different amines with the acid chloride 2-[4-(2-chlorocarbonyl-ethoxy)-phenyl]-4-methyl-thiazole-5-carboxylic acid ethyl ester (**3**) gave moderate yields in the presence of triethylamine as acid scavenger but with N-methylimidazole as catalyst gave high yields[24] (75-85%). All the newly synthesized compounds were characterized by IR, NMR and mass spectral analysis.

Amide carbonyl group stretching band is observed in the region 1620-1665 cm^{-1}. Absorption band in the region 3180-3350 cm^{-1} corresponds to the NH stretching for the compounds **4(e-j)** and no absorption band in this region for the compounds **4**(**a-d**) because, lack of NH bond in that compounds[28-31]. ^{1}H NMR showed signal at δ 2.40-4.83 indicates the methylene protons and signals due to NH protons were observed in the region δ 9.54-10.16 ppm.[32-35] Signals in the range of δ 6.42-8.54 ppm correspond to aromatic protons. In ^{13}C NMR spectra signals at δ 32.2-65.6 ppm confirmed the presence of methylene carbons and peaks in the region at δ 167.3-170.6 ppm indicates the carbonyl group of amide and ester. LCMS were recorded for a few representative compounds, they gave $M^{+\cdot}$ ions at their respective molecular masses. C, H and N analysis was obtained for a few title compounds and the data confirmed their elemental composition.

3. Antimicrobial activity

Antimicrobial activity was evaluated by measuring the Zone of inhibition against the tested organisms and the results are summarized in **Table 1** (Antibacterial) and **Table 2** (Antifungal). Each test was carried out three times and average values are taken. All the compounds were studied for their *in vitro* antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. Among the tested compounds **4c, 4f, 4g** and **4i** showed better activity than that of others, it might be due to the presence of Cl and F substitution at different positions. Fluorine containing compounds **4c, 4g** and **4i** showed good inhibition activity against all the strains tested. The compound **4g** having two F atoms at positions 4 and 6 on benzothiazole ring, exhibited the highest activity. Compound **4a** has shown good inhibition due to the presence of NO_2 group.

The synthesized compounds were also screened for their antifungal activity against *Trichoderma viride*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum* by disc diffusion method at 100 µg/mL concentration. Compounds **4c** and **4g** showed highest zone of inhibition when compared to others against the tested fungal strains.

Table1. Antibacterial activity of synthesized compounds **4(a-j)**

Entry	Product	Zone of Inhibition in mm			
		P. aeruginosa	*K. pneumoniae*	*E. coli*	*S. aureus*
1	**4a**	26	25	28	30
2	**4b**	16	20	18	22
3	**4c**	28	26	30	32
4	**4d**	24	28	25	30
5	**4e**	29	30	29	24
6	**4f**	30	28	31	26
7	**4g**	38	34	32	36
8	**4h**	22	24	20	18
9	**4i**	26	24	28	30
10	**4j**	18	20	16	14
11	**Stand**	42	38	36	40

Concentration at 100 µg/mL. *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli: Escherichia coli*, *S. aureus: Staphylococcus aureus*, Stand: *Chloramphenicol*

Table 2. Antifungal activity of synthesized compounds **4(a-j)**

Entry	Product	Zone of Inhibition in mm			
		A.niger	*T. viride*	*A. flavus*	*P. chrysogenum*
1	**4a**	16	12	10	14
2	**4b**	12	10	9	11
3	**4c**	18	20	10	12
4	**4d**	12	16	11	9
5	**4e**	17	15	12	14
6	**4f**	16	18	11	13
7	**4g**	24	16	12	16
8	**4h**	14	12	8	10
9	**4i**	20	18	9	13
10	**4j**	15	8	10	9
11	**Stand**	26	22	16	20

Concentration at 100 µg/mL. *A.niger: Aspergillus niger, T. viride: Trichoderma viride, A. flavus*: *Aspergillus flavus, P. chrysogenum: Penicillium chrysogenum,* Stand: nystatin.

4. Experimental Section

Required chemicals were purchased from Sigma-Aldrich Chemicals. Melting points were determined by using a calibrated thermometer Guna digital melting point apparatus and are uncorrected. The FT-IR spectra were recorded using ALPHA (Bruker). ^{1}H and ^{13}C NMR were recorded on Bruker AMX 400 MHz by using $CDCl_3$ as solvent and TMS as an internal standard. Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck-made TLC plates. Liquid chromatography (LC) mass spectra were recorded on a Shimadzu LCMS 2010A.

4.1. General procedure for the synthesis of 2-[4-(2-carboxy-ethoxy)-phenyl]-4-methyl-thiazole-5-carboxylic acid ethyl ester (2)

Sodium hydride (0.002 mole) in THF was added to 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (**1**) (0.001 mole) in 5 mL of pyridine and 10 mL of THF, stirred for 1h at 5-20 °C. 3-Bromopropanoic acid (0.001 mole) in THF was added dropwise to phenoxide, stirred for 1 h at 10-45 °C. It was filtered to get crude compound (**2**) and evaporated the solvent under vacuum, washed with hexane and ethyl acetate.

4.2. General procedure for the synthesis of ethyl 2-(4-(3-chloro-3- oxopropoxy) phenyl)-4-methylthiaz ole-5-carboxylate (3)

To a stirred solution of acid (**2**) (0.001 mole) in dry THF (10 mL), excess of thionyl chloride (0.0015 mole) was added at 0 °C in the presence of Et_3N and stirred for 1 h at 30 °C to afford ethyl 2-(4-(2-(chlorocarbonyl)ethoxy)phenyl)-4-ethylthiazole-5-carboxylate (**3**). $Et_3N.HCl$ was removed by filtration, the solvent and unreacted thionyl chloride was removed in a rotaevaporator.

4.3. General procedure for the synthesis carboxamides (4a-j)

The acid chloride (**3**) is reacted with various bioactive amines in the presence of N-methylimidazole as a base and THF as solvent at 10-30 °C. The progress of the reaction was monitored by TLC (n-Hexane: Ethyl acetate 3:1). After completion of the reaction, water was added to the stirred mixture, which was extracted with ethyl acetate. The organic layer was washed with 5% HCl solution

and 10% $NaHCO_3$ solution in order to remove unreacted amine and acid respectively. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated in a rotaevaporator. The obtained crude product was purified by silica gel column chromatography to obtain respective amides. The physical properties and spectral data of the obtained compounds are given below.

4.3.1.3-(4-(5-(Ethoxycarbonyl)-4-methylthiazol-2-yl)phenoxy)propanoic acid (2): White solid, yield 85%; mp: 211-212 °C; IR (cm^{-1}): 3420, 1715, 1670; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (t, J = 7.0 Hz, 3H), 2.34 (s, 3H), 2.42 (t, J = 7.1 Hz, 2H), 3.46 (t, J = 7.0 Hz, 2H), 4.34 (q, J = 7.2 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H, Ar-H), 7.42 (d, J = 8.6 Hz, 2H, Ar-H), 10.52 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.3, 18.5, 35.6, 61.4, 67.5, 115.4 (2C), 126.8, 127.2, 128.6 (2C), 158.6, 161.4, 165.8, 169.5, 170.6; MS: 335 ($M^{+\cdot}$, 80) 265 (30), 156 (100); Anal. Calcd for $C_{16}H_{17}NO_5S$: C, 57.48; H, 5.87; N, 4.66. Found C, 57.12; H, 5.23; N, 4.14.

4.3.2. Ethyl 2-(4-(3-(4-(4-nitrophenyl)piperazin-1-yl)-3-oxopropoxy)phenyl)-4-methylthiaz ole-5-carboxylate (4a): Yellow solid, yield 92%; mp: 225-226 °C; IR (cm^{-1}): 2980, 1725, 1620; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, J =7.2 Hz, 3H), 2.40 (s, 3H), 2.65 (t, J = 7.0 Hz, 2H), 3.28 (t, J = 7.1 Hz, 2H), 3.48 (t, J = 7.2 Hz, 2H), 4.28 (t, J = 7.0 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H, Ar-H), 7.42 (d, J = 8.4 Hz, 2H, Ar-H), 7.64 (d, J = 8.4 Hz, 2H, Ar-H), 8.12 (d, J = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.6, 16.2, 33.6, 44.2 (2C), 48.6 (2C), 62.5, 65.6, 113.5 (2C), 116.4 (2C), 124.5, 125.8 (2C), 127.4, 129.5 (2C), 138.6, 155.5, 158. 5, 160.5, 161.8, 167.5, 168.7; MS: 524 ($M^{+\cdot}$, 75), 467 (35), 263 (100); Anal. Calcd for $C_{26}H_{28}N_4O_6S$: C, 59.53; H, 5.38; N, 10.68. Found C, 59.12; H, 5.08; N, 10.24.

4.3.3. Ethyl 2-(4-(3-oxo-3-(4-(pyridin-2-yl)piperazin-1-yl)propoxy)phenyl)-4-methylthiazole-5-carboxylate (4b): White solid, yield 85%; mp: 205-206 °C; IR (cm^{-1}): 2970, 1720, 1628; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, J = 7.1 Hz, 3H), 2.44 (s, 3H), 2.65 (t, J = 7.0 Hz, 2H), 3.18 (t, J = 7.2 Hz, 2H), 3.48 (t, J = 7.0 Hz, 2H), 4.28 (t, J = 7.1 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 6.58-7.65 (m, 3H, Ar-H), 7.18 (d, J = 8.6 Hz, 2H, Ar-H), 7.32 (d, J = 8.6 Hz, 2H, Ar-H), 8.22 (d, J = 8.8 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.8, 16.3, 32.6, 45.5 (2C), 47.5 (2C), 64.6, 63.5, 110.4 (2C), 112.5 (2C), 126.3, 127.5 (2C), 131.3, 136.8, 151.4, 155.5, 158.5, 161.4, 165.8, 167.5, 169.1; MS: 480 ($M^{+\cdot}$, 65), 342 (45), 184 (100); Anal. Calcd for $C_{25}H_{28}N_4O_4S$: C, 62.48; H, 5.87; N, 11.66. Found C 62.12; H 5.38; N 11.34.

4.3.4. Ethyl 2-(4-(3-(4-(4-fluorophenyl)piperazin-1-yl)-3-oxopropoxy)phenyl)-4-methylthiaz ole-5-carboxylate (4c): White solid, yield 90%; mp: 215-216 °C; IR (cm^{-1}): 2980, 1732, 1665; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, J = 7.1 Hz, 3H), 2.40 (s, 3H), 2.55 (t, J = 7.2 Hz, 2H), 3.18 (t, J = 7.0 Hz, 2H), 3.78 (t, J = 7.0 Hz, 2H), 4.25 (t, J = 7.1 Hz, 2H), 4.46 (q, J = 7.0 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H, Ar-H), 7.22 (d, J = 8.4 Hz, 2H, Ar-H), 7.35 (d, J = 8.4 Hz, 2H, Ar-H), 7.58 (d, J = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.4, 17.8, 32.2, 46.6 (2C), 47.5 (2C), 57.4, 68.4, 115.5 (2C), 117.8 (2C), 118.5 (2C), 125.7, 127.5, 129.5 (2C), 143.2, 155.5, 157.6, 162.3, 165.6, 167.4, 169.7; MS: 497 ($M^{+\cdot}$, 35), 469 (58), 235 (100).

4.3.5. Ethyl 2-(4-(3-oxo-3-(4-(pyrimidin-2-yl)piperazin-1-yl)propoxy)phenyl)-4-methylthiaz ole-5-carboxylate (4d): White solid, yield 78%; mp: 202-204 °C; IR (cm^{-1}): 2980, 1718, 1645; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, J = 7.2 Hz, 3H), 2.40 (s, 3H), 2.45 (t, J = 7.0 Hz, 2H), 3.30 (t, J = 7.1 Hz, 2H), 3.68 (t, J = 7.2 Hz, 2H), 4.28 (t, J = 7.1 Hz, 2H), 4.32 (q, J = 7.2 Hz, 2H), 6.84 (dd, J = 2.4 and 8.6 Hz, 1H, Ar-H), 7.24 (d, J = 8.4 Hz, 2H, Ar-H), 7.48 (d, J = 8.4 Hz, 2H, Ar-H), 8.44 (d, J = 8.6 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.8, 16.7, 34.4, 45.6 (2C), 48.5 (2C), 58.5, 66.4, 108.8, 113.5 (2C), 115.6, 126.5, 129.5 (2C), 135.4, 156.2, 158.8 (2C), 160.6, 161.7, 168.2, 169.4; MS: 481 ($M^{+\cdot}$, 58), 436 (55), 376 (100); Anal. Calcd for $C_{24}H_{27}N_5O_4S$: C, 59.86; H, 5.65; N, 14.54. Found C, 59.42; H, 5.32; N, 14.34.

4.3.6. Ethyl 2-(4-(2-(benzo[d]thiazol-2-ylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4e): White solid, yield 84%; mp: 218-219 °C; IR (cm^{-1}): 3270, 1724, 1655; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, J = 7.1 Hz, 3H), 2.40 (s, 3H), 2.65 (t, J = 7.2 Hz, 2H), 4.12 (t, J = 7.1 Hz, 2H), 4.32 (q, J = 7.0 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H, Ar-H), 7.56 (d, J = 8.6 Hz, 2H, Ar-H), 7.62-8.24 (m, 4H, Ar-H), 9.54 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.7, 17.4, 32.5, 58.5, 66.8, 116.2 (2C), 118.2, 120.6, 124.5, 125.5, 127.3, 129.8 (2C), 130.4, 134.8, 150.6, 158.2, 160.6, 161.3, 167.4, 169.6, 170.2; MS: 467 ($M^{+\cdot}$, 60), 423 (45), 235 (100); Anal. Calcd for $C_{23}H_{21}N_3O_4S_2$: C, 59.38; H, 4.53; N, 8.99. Found C, 59.08; H, 4.18; N, 8.34.

4.3.7. Ethyl 2-(4-(2-(4-chlorobenzo[d]thiazol-2-ylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4f): White solid, yield 90%; mp: 230-231 °C. IR (cm^{-1}): 3271, 1716, 1641; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, J = 7.2 Hz, 3H), 2.44 (s, 3H), 3.61 (t, J = 7.1 Hz, 2H), 4.08 (t, J = 7.0 Hz, 2H), 4.21 (q, J = 7.2 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H, Ar-H), 7.34 (d, J = 8.6 Hz, 2H, Ar-H), 7.55-7.84 (m, 3H, Ar-H), 10.16 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.5, 17.7, 35.2, 61.3, 63.2, 116.4, 119.5, 120.5 (2C), 121.7 (2C), 121.9, 123.8, 125.9, 126.5, 127.3, 128.8, 132.5, 149.8, 160.5, 161.9, 167.7, 169.8; MS: 501 ($M^{+\cdot}$, 54), 466 (48), 337 (100); Anal. Calcd for $C_{23}H_{20}ClN_3O_4S_2$: C, 55.63; H, 4.94; N, 8.98. Found C, 55.12; H, 4.73; N, 8.84.

4.3.8. Ethyl 2-(4-(2-(4,6-difluorobenzo[d]thiazol-2-ylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4g): White solid, yield 88%; mp: 236-237 °C; IR (cm^{-1}): 3470, 1728, 1645; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J = 7.1 Hz, 3H), 2.36 (s, 3H), 2.42 (t, J =7.0 Hz, 2H), 3.64 (t, J =7.2 Hz, 2H), 4.32 (q, J =7.1 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.45 (d, J=8.4 Hz, 2H, Ar-H), 7.82 (s, 1H, Ar-H), 10.12 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.6, 16.5, 34.7, 59.5, 63.5, 98.5, 104.2, 105.6, 114.5 (2C), 125.6, 127.5, 128.8 (2C), 152.4, 157.3, 158.5 (2C), 160.6, 161.5, 165.5, 167.2, 169.6; MS: 503 ($M^{+\cdot}$, 45), 419 (34),341 (100).

4.3.9. Ethyl 2-(4-(2-((pyridin-3-yl)methylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4h): Pale Yellow solid, yield 76%; mp: 195-196 °C; IR (cm^{-1}): 3250, 1718, 1648; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32 (t, J =7.0 Hz, 3H), 2.40 (s, 3H), 2.48 (t, J =7.2 Hz, 2H), 3.88 (t, J = 7.0 Hz, 2H), 4.32 (q, J = 7.2 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H, Ar-H), 7.52 (d, J = 8.6 Hz, 2H, Ar-H), 7.52-8.34 (m, 3H, Ar-H), 8.54 (s, 1H, Ar-H), 9.78 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8, 17.8, 35.6, 43.5, 61.4, 65.6, 113.5 (2C), 122.5, 124.5, 127.5, 128.8 (2C), 133.5, 135.3, 146.5, 152.5, 158.4, 161.5, 165.5, 168.5, 169.3; MS: 425 ($M^{+\cdot}$, 54), 341 (55), 262 (100).

4.3.10. Ethyl 2-(4-(2-(2-chlorobenzylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4i): White solid, yield 70%; mp: 229-230 °C; IR (cm^{-1}): 3240, 1712, 1636; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, J = 7.1 Hz, 3H), 2.35 (s, 3H), 2.55 (t, J = 7.0 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 4.16 (d, J = 6.8 Hz, 2H), 4.32 (t, J = 7.0 Hz, 2H), 4.78 (s, 2H), 6.94 (d, J = 8.4 Hz, 2H, Ar-H), 7.56 (d, J = 8.4 Hz, 1H, Ar-H), 7.14-7.62 (m, 4H, Ar-H), 9.82 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8, 16.5, 34.8, 35.8, 62.5, 65.5, 115.5, 124.5, 125.5, 126.8, 127.6 (2C), 128.7, 128.5 (2C), 129.3, 131.5, 141.2, 158.4, 161.6, 164.5 167.3, 169.5; MS: 458 ($M^{+\cdot}$, 58), 421 (58), 335 (100).

4.3.11. Ethyl 2-(4-(2-(4-fluorobenzylcarbamoyl)ethoxy)phenyl)-4-methylthiazole-5-carboxylate (4j): Pale Yellow solid, yield 72%; mp: 221-222 °C; IR (cm^{-1}): 3280, 1717, 1655; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.18 (t, J = 7.2 Hz, 3H), 2.40 (s, 3H), 2.65 (t, J = 7.0 Hz, 2H), 4.28 (t, J = 7.1 Hz, 2H), 4.32 (q, J = 7.2 Hz, 2H), 4.46 (d, J = 6.8 Hz, 2H), 4.83 (s, 2H), 6.96 (d, J = 8.4 Hz, 2H, Ar-H), 7.12 (d, J = 8.6 Hz, 2H, Ar-H), 7.42 (d, J = 8.6 Hz, 2H, Ar-H), 7.44 (d, J = 8.4 Hz, 2H, Ar-H), 9.92 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.5, 14.5, 33.5, 43.2, 59.5, 64.2, 113.5, 115.4 (2C), 116.6 (2C), 125.5, 127.4, 128.9 (2C), 128.5 (2C), 138.5, 159.5, 161.5, 165.5, 167.5, 169.2; MS: 442 ($M^{+\cdot}$, 68), 342 (44), 263 (100).

5. Antimicrobial activity:

All the title compounds were dissolved in DMSO and evaluated for their antimicrobial activities *in vitro* by disc diffusion method. Bacterial cultures were prepared in Nutrient Agar medium (NAM) and for fungal test Potato Dextrose Agar (PDA) medium was used. 10 mL of distilled water was used to scrape conidia from 10 days culture and the spores were collected after filtration. The spores were resuspended in sterile distilled water and were used as inoculum. For bacterial culture plates a 100 µL of the cell suspension (10^6 cells/ mL) was used to prepare bacterial lawn. Antimicrobial tests were done by disc diffusion technique.[35,36] Discs were prepared with Whatman No.1 filter paper (6 mm diameter) and was impregnated with 100 µg/ disc of each compound and placed on the inoculated microbial plates. And all the plates were subjected to incubation at 37 $^{\circ}$C for 24 hours for bacterial culture and 48-72 hours for fungal culture. Chloramphenicol was used as positive control and was placed in the centre of all the plates for bacterial cultures and nystatin was used as positive control for fungal cultures.

6. Conclusion

A convenient method was developed for the synthesis of biologically active carboxamide derivatives of 3-(4-(ethoxycarbonyl)-4-methylthiazol-2yl)phenoxy)propanoic acid. The newly synthesized compounds exhibited good antimicrobial activity against tested strains. It was noticed that among the synthesized compounds **4c**, **4g** and **4i** showed significant inhibition against both bacteria and fungi when compared to that of standards.

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