ORIGINAL ARTICLE

A C G publications

Org. Commun. 9:3 (2016) 54-64

organic communications

Design, synthesis, characterization and bioassay of novel carboxamide derivatives of Celecoxib

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(Received April 11, 2016; Revised June 27, 2016; Accepted October 4, 2016)

Abstract: A series of new carboxamide derivatives of celecoxib, 4-(5-*p*-tolyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide **1** were synthesized by the reaction of 3-(4-(3-(trifluoromethyl)-5-*p*-tolyl-1*H*-pyrazol-1-yl)phenylsulfonylamino)propanoic acid **2** with various bio-potent amines by using 1-methylimidazole as a base *via*. Schotten-Baumann reaction. The acid **2,** which inturn prepared from **1** by treating with 3-bromopropanoic acid in the presence of sodium hydride. The newly synthesized compounds were characterized by IR, NMR and mass spectral analysis. The title molecules were evaluated for their efficacy as antimicrobial and antioxidant agents *in vitro*. Compounds **4(c-g)** showed high growth inhibitory activity against both bacteria and fungi. The compounds **4c** and **4f** exhibited promising antioxidant activity.

Keywords: Carboxamide; 1-methyl imidazole; Schotten-Baumann reaction; antioxidant activity; antimicrobial activity.

1. Introduction

A heterocyclic scaffold possesses diverse biological activity in various therapeutic areas like anti-inflammatory, antipyretic, analgesic, antimicrobial, anticonvulsant and antihypertensive agents. Numerous azole classes of compounds, mainly pyrazoles and their derivatives constitute an important class of heterocyclic motifs because of their prevalent applications in medicinal chemistry, agrochemical[1,2] and pharmaceutical industries[3]. They are associated with diverse pharmacological activities such as antibacterial[4], antioxidant[5,6], anti-HIV[7], anti-inflammatory[8] and anticancer[9]. This contributed a great impetus to the exploration for potential pharmacologically active drugs carrying pyrazole derivatives.

The chemistry of amide bond plays a vital role in compounds of pharmaceutical, chemical and natural sectors which has grabbed intensive attention from all over the world because of its medicinal importance. Amide linkage is the backbone of proteins. Besides, amide derivatives displayed excellent biological properties viz. antibacterial, antiproliferative[10], antioxidant[11,12], analgesic, anti-inflammatory[13], antitumor[14], cyclooxygenase inhibitors[15] and xanthine oxidase inhibitory activity[16]. Amide has three bioisosters like normal acid amide, sulfonamide and phosphoramide and each one has its own pharmacological properties. Among them sulfonamide pharmacophore has conceivedimportance in medicinal chemistry owing to its immense biological properties. It is the core unit in many well-known drugs like Darunavir (protease inhibitor) and Celecoxib (COX-2 inhibitor).

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/OC/index.htm © Published 10/01/2016 EISSN:1307-6175

Further, pyrazole containing analogues are considered as cyclooxygenase inhibitors. Non-Steroidal anti-inflammatory drugs (NSAIDs) are used to inhibit the cyclooxygenase, which causes pain and inflammation. Most of the non-steroidal anti-inflammatory drugs have gastrointestinal (GI) and renal side effects[17-19]. General NSAIDs such as aspirin, ibuprofen and Diclofenac are non-selective, show greater selectivity for COX-1, which is responsible for the cytoprotection in the GI tract whereas the selective COX-2 inhibitor, a second generation of non-steroidal anti-inflammatory drug (NSAID) which has no side effects of gastrointestinal (GI) damage[20]. Celcoxib is the second generation NSAID and COX-2 selective with lower side effects. In addition, Cimicoxib, Valdecoxib and Naproxen were also used as COXIBs, which are shown in Figure 1.

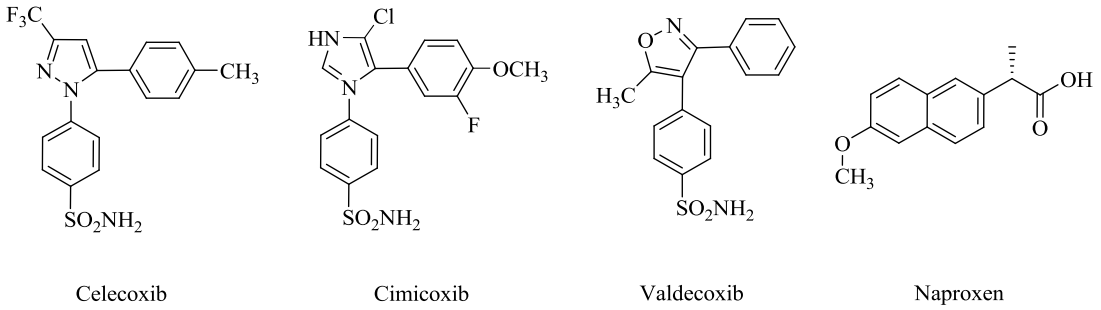

Figure 1. Some anti-inflammatory drugs

Celecoxib has three bioisosters *i.e.,* aryl group, sulfonamide group and heterocycle/carbocycle, Figure 2 which are responsible for the anti-inflammatory activity via a hydrogen bonding interaction[21,22]. Varied biological and pharmacological activities of the celecoxib derivatives enhanced by structural modification were reported by many workers[23-25]. Hellberg, *et al* reported that the amide derivatives of non-steroidal anti-inflammatory drug Naproxen displayed good antioxidant activity [26]. The search for novel antimicrobial and antioxidant agents devoid of side effects continues to be an active area of research in medicinal chemistry.

In view of the above cited findings, herein we report the synthesis of new acid amide derivatives of celecoxib by structural modification at bioisoster II *i.e.,* sulfonamide by treating with 3-bromopropanoic acid, evaluated for their efficacy as antimicrobial and antioxidant agents.

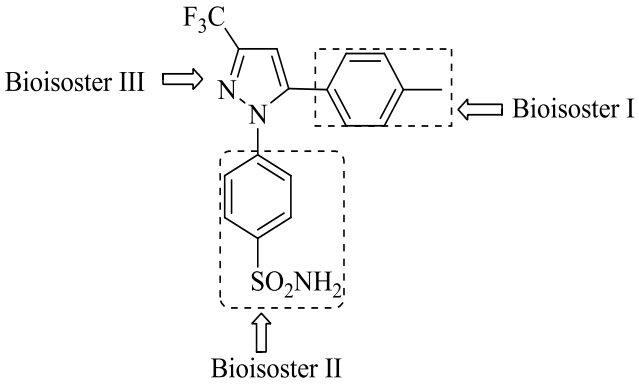

Figure 2. Bioisosters of Celecoxib

2. Results and discussion

2.1. Chemistry

The synthetic pathway to the targeted compounds **4**(**a**-**h**) was sketched in **Fig. 3**. In brief, commercially available Celecoxib (**1**) was used as starting material. It was converted into propanoic acid **2** by the sodium hydride mediated reaction with 3-bromopropanoic acid in THF. Acid chloride **3**

was synthesized by reaction of the acid **2** with $SOCl_2$, which in turn reacted with various eight bio-active amines in the presence of NEt_3 or N-methylimidazole[27-29], in THF at 10-30 ºC to give the corresponding amides **4a-h**.

While NEt_3 catalyzed reactions gave the amides with low yields, the use of *N*-methylimidazole[30] increased the yields to moderate (76-88%). All the newly synthesized amides were characterized by IR, NMR and mass spectral analysis. Amide carbonyl group stretching band is observed in the region 1616–1648 cm^{-1}. A band in the region 3228-3252 cm^{-1} corresponds to the amide NH stretching for the compounds **4**(**a-d**). Absorption bands due to SO_2NH were observed at 3220-3248 cm^{-1} and symmetric, asymmetric bands for SO_2 were observed at 1122-1154 cm^{-1} and 1320-1348 cm^{-1}.

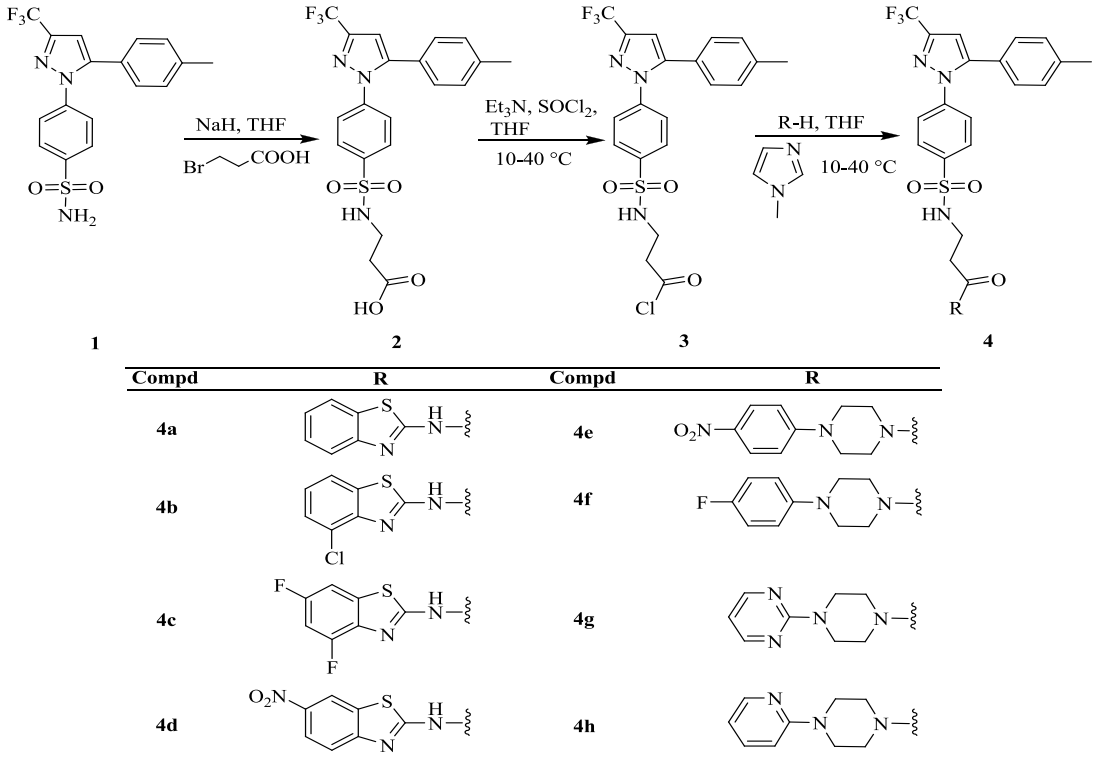

Figure 3. Synthesis of amide derivatives of celecoxib

In ^1H NMR, a signal at δ 2.42-4.48 indicates the methylene protons and signals due to amide NH protons were observed in the region δ 10.96-11.25 for the compounds **4**(**a-d**). Signals in the range of δ 6.28- 8.92 correspond to aromatic protons and the signals at δ 10.24-10.48 are due to SO_2NH protons. In ^{13}C NMR spectra, signals δ 34.2-38.5 confirmed the presence of methylene carbons and peaks in the region at δ 169.6- 173.7 indicate the carbonyl group of amide. EIMS were recorded for a few representative compounds; they gave M+H ions at their respective molecular masses. CHN 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). All the target compounds exhibited promising antibacterial and antifungal activities. The title compounds were dissolved in DMSO at 100 µg/ mL and each test was carried out three times and mean values of inhibition zone diameter are taken. All the synthesized compounds were screened for their in vitro antibacterial activity against the following bacterial strains: Gram negative bacteria *Escherichia coli* and *Klebsiella pneumonia*, gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Moderate to excellent antibacterial activity was disclosed by the synthesized analogs. Among the

synthesized amides **4c-4g** showed increased antibacterial activity against the growth of E. coli and S. aureus than that of others compared to standard Chloramphenicol. When we compare the antibacterial activity of the compounds, it is cleared that the effect of substitution plays major role in inhibition of the growth of the bacterial and fungal strains. The antibacterial activity of the compounds 4a-4d against tested bacterial strains is as follows, **4a** < **4b** < **4d** < **4c**. Compounds **4b-4d** displayed more antibacterial activity than **4a** due to the presence of substitution in the former ones and lacks in the latter compound. Substitutions like halogens (Cl, F) and nitro group at 4, 6 positions on the benzene ring in the compounds **4b-4d** and *p*-position on the phenyl ring in the case of compounds **4e** and **4f** enhances the zone of inhibition. Again compound **4c** showed more antibacterial activity with MIC (3.25 μg/ mL) than **4b** (6.75 μg/ mL), due to fluorine substitution[31] which enhances the activity. Similar trend is observed in the case of **4e-4h** analogs, **4h < 4g < 4e < 4f**. The zone of inhibition of the compound **4h < 4g**, due to the presence of pyrimidine ring in **4g**.

Table 1. Antibacterial activity of synthesized compounds **4(a-h)**

Entry	Product	Gram negative				Gram positive			
		E. coli		*K. pneumoniae*		*B. subtilis*		*S. aureus*	
		IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
1	**4a**	25	8.45	17	10.64	29	9.85	27	10.42
2	**4b**	28	6.74	20	7.85	32	3.84	30	8.15
3	**4c**	32	3.26	26	5.24	35	1.65	34	1.35
4	**4d**	30	5.15	22	8.24	33	5.15	31	7.85
5	**4e**	26	11.45	24	11.45	32	8.75	28	9.45
6	**4f**	29	13.34	25	9.35	34	6.25	32	6.14
7	**4g**	25	15.15	21	14.44	30	12.35	26	11.45
8	**4h**	23	16.68	20	19.16	28	17.35	24	18.28
9	**Std**	34	6.25	28	12.35	38	8.34	36	10.25

E. coli: Escherichia coli, K. pneumoniae: *Klebsiellapneumoniae, B. subtilis: Bacillus subtilis, S. aureus*: *Staphylococcus aureus*, Stand: Chloramphenicol
IZ: Inhibition zone in (mm), Concentration at 100 μg/mL
MIC: Minimum inhibitory concentration (in μg/mL).

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

Entry	**Product**	***A. niger***		***T. viride***		***A. flavus***		***P. chrysogenum***	
		IZ	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**
1	**4a**	17	8.45	23	9.45	20	7.85	14	9.45
2	**4b**	20	2.75	25	6.75	22	3.24	16	6.25
3	**4c**	23	1.85	31	3.14	26	2.65	19	4.35
4	**4d**	21	2.15	27	5.64	24	4.25	17	7.25
5	**4e**	19	2.65	26	7.15	23	5.75	18	8.45
6	**4f**	22	2.35	29	5.25	25	4.55	16	7.24
7	**4g**	18	7.45	24	10.25	21	10.35	15	12.34
8	**4h**	17	9.55	22	15.36	19	15.45	13	16.48
9	**Std**	25	4.25	32	8.45	28	6.44	22	8.25

A. niger: *Aspergillus niger, T. viride*: *Trichoderma viride,*
A. flavus: *Aspergillus flavus, P. chrysogenum: Penicillium chrysogenum,*
IZ: Inhibition zone in (mm), Concentration at 100 μg/mL
MIC: Minimum inhibitory concentration (in μg/mL), standard: Nystatin

On the other hand, study of the antifungal activity of the synthesized compounds revealed that all the analogs exhibited substantial growth inhibitory activity against *Trichoderma viride*, *Aspergillus*

niger, *Aspergillus flavus* and *Penicillium chrysogenum* by disc diffusion method at 100 µg/mL concentration. Nystatin was used as a standard antifungal agent. The analogs showed high activity towards *Trichoderma viride* and *Aspergillus flavus*. In particular, compounds **4c** and **4f,** (MIC 3.14 and 5.25 µg/mL) displayed highest zone of inhibition when compare to standard antifungal agent against *Trichoderma viride*.

3.1. Antioxidant activity

The radical scavenging activity of the title compounds was carried out by DPPH method and Superoxide radical scavenging method at two different concentrations 50 and 100 µg/mL. Most of the title compounds exhibited good antioxidant activity when compared to natural antioxidant ascorbic acid as a standard. Especially compounds **4c**, **4d**, **4e** and **4f** displayed excellent radical scavenging activity in super oxide scavenging method due to the presence of Cl, F and NO_2 groups at *p*-positions in the case of **4e** and **4f**. The order of antioxidant activity was **4c > 4d > 4e > 4f** with 85.45 > 82.22 > 78.32 > 76.24 percent of inhibition respectively. The rest of the compounds showed moderate antioxidant activity.

Table 3. Antioxidant activity of synthesized compounds **4(a-h).**

Entry	Product	DPPH Scavenging (%)		Superoxide Scavenging (%)	
		50 µg/ mL	**100 µg/ mL**	**50 µg/ mL**	**100 µg/ mL**
1.	**4a**	48.82±1.16	54.45±1.16	58.84±1.23	71.28±1.16
2.	**4b**	55.52±1.14	62.34±1.18	66.25±1.15	76.42±1.20
3.	**4c**	62.34±1.13	71.42±1.28	73.24±1.14	85.45±1.16
4.	**4d**	61.18±1.16	68.14±1.12	71.54±1.26	82.22±1.12
5.	**4e**	57.42±1.08	64.54±1.04	68.64±1.12	78.32±1.04
6.	**4f**	59.14±1.22	66.34±1.14	67.42±1.08	76.24±1.18
7.	**4g**	53.64±1.15	61.25±1.22	61.56±1.13	69.64±1.08
8.	**4h**	45.42±1.23	53.74±1.05	54. 84±1.20	62.84±1.10
9.	**Std**	64.24±1.16	72.46±1.12	74.42±1.10	86.34±1.08

Values obtained by the mean of three replicates ± SD, Standard: Ascorbic acid

4. Experimental Section

Chemicals were purchased from Sigma-Aldrich Chemicals. Melting points were determined on 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 DMSO-d_6 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. CHN analysis was performed with the Thermo Finnigan Flash EA 1112 instrument at the IICT, Hyderabad, India.

4.1.1. General procedure for the synthesis of 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)propanoic acid (2)

NaH (2 mmol) in THF (10 mL) was added to celecoxib (**1**) (1 mmol) in pyridine (5 mL) and THF (10 mL), stirred for 1h at 5-20^{o}C. 3-Bromopropanoic acid (1 mmol) in THF (10 mL) was added dropwise to the mixture, stirred for 1h at 10-45^{o}C. The crude compound (**2**) was filtered, evaporated, and washed with hexane to give 2.

4.1.1.2. General procedure for the synthesis of compound (3)

To a stirred solution of crude acid **2** (0.001 mol) in dry THF (10 mL), excess of $SOCl_2$ (0.0015 mol) was added at 0°C in the presence of Et_3N (0.001 mole) and stirred for 1 h at 30°C. $Et_3N.HCl$ was removed by filtration, the solvent and unreacted $SOCl_2$ was removed under reduced pressure to give crude propanoyl chloride **3**.

4.2. General procedure for the synthesis of Carboxamides 4(a-h)

To a stirred solution of crude acid chloride **3** in dry THF (10 mL), various bioactive amines (1 mmol) in the presence of N-methylimidazole (2 mL) as a base were added at 10°C. After the addition of amine, reaction temperature was raised to 30-40°C and stirred the reaction mixture until the completion of the reaction. 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 then 10% $NaHCO_3$ solution. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated in a rotaevaporator. The 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-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)propanoic acid (2): White solid, yield 88%; mp 211-212; IR (cm^{-1}): 3410 (COOH), 3230 (SO_2NH), 1705 (C=O), 1315, 1135 (SO_2). ^1H (DMSO-d_6) δ: 2.18 (s, 3H), 2.77 (t, J = 4.5 Hz, 2H), 3.66 (t, J = 5.2 Hz, 2H), 6.36 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H, Ar-H), 7.42 (d, J= 8.0 Hz, 2H, Ar-H), 7.68 (d, J = 8.4 Hz, 2H, Ar-H), 8.22 (d, J = 8.4 Hz, 2H, Ar-H), 10.48 (s, 1H, SO_2NH), 11.12 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 25.8, 34.8, 36.6, 107.4, 120.4 (2C), 125.7 (2C), 126.4 (2C), 128.5 (2C), 132.7, 132.4, 137.6, 139.5, 142.5, 144.2, 148.4, 173.6. MS: 453 (M +H).

4.3.2. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-N-(benzo[d] thiazol-2-yl)propanamide (4a): White solid, yield 90%; mp 241-242; IR (cm^{-1}): 3248 (SO_2NH), 3228 (CONH), 1630 (C=O), 1335, 1144 (SO_2). ^1H (DMSO-d_6) δ: 2.25 (s, 3H), 2.74 (t, J = 7.2 Hz, 2H), 4.42 (t, J = 7.0 Hz, 2H), , 6.48 (s, 1H), 7.14 (d, J = 8.0 Hz, 2H, Ar-H), 7.31 (d, J = 8.4 Hz, 2H, Ar-H), 7.40 (d, J = 8.6 Hz, 2H, Ar-H), 7.64- 8.14 (m, 4H, Ar-H), 8.26 (d, J = 8.6 Hz, 2H, Ar-H), 10.46 (s, 1H, SO 2NH), 11.15 (brs, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ: 25.4, 34.6, 36.2, 106.4, 119.4 (2C), 120.6 (2C), 122.5, 124.6 (2C), 126.4, 127.5, 128.7 (2C), 129.5 (2C), 132.4, 134.2, 138.3, 142.2, 143.5, 145.4, 147.6, 155.8, 167.4, 172.6. MS: 585 (M+H). Anal. Calcd for $C_{27}H_{22}F_3N_5O_3S_2$: C, 55.78; H, 3.87; N, 11.76. Found C, 55.32; H, 3.53; N, 11.44.

4.3.3. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-N-(4-chlorobenzo[d]thiazol-2-yl)propanamide (4b): White solid, yield 92%; mp 258-259; IR (cm^{-1}): 3234 (SO_2NH), 3265 (CONH), 1636 (C=O), 1325, 1128 (SO_2). ^1H (DMSO-d_6) δ: 2.22 (s, 3H), 2.64 (t, J = 7.2 Hz, 2H), 3.32 (t, J = 7.0 Hz, 2H), 6.34 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H, Ar-H), 7.28 (d, J = 8.4 Hz, 2H, Ar-H), 7.36-7.42 (m, 1H, Ar-H), 7.58 (d, J = 8.4 Hz, 1H, Ar-H), 7.68 (d, J = 8.0 Hz, 2H, Ar-H), 7.94 (d, J = 8.4 Hz, 2H, Ar-H), 8.14 (d, J = 8.8 Hz, 1H, Ar-H) 10.42 (s, 1H, SO_2NH), 11.20 (brs, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ: 25.8, 33.7, 37.5, 104.5, 119.5, 120.4, 122.2, 123.6, 124.3 (2C), 125.2, 126.7 (2C), 128.2 (2C), 129.2 (2C), 132.5, 135.7, 137.2, 141.7, 143.7, 145.3, 149.7, 156.2, 171.4, 173.6. MS: 619 (M+H).

4.3.4. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-N-(4,6-difluorobenzo[d]thiazol- 2-yl)propanamide (4c): White solid, yield 94%; mp 248-249; IR (cm^{-1}): 3224 (SO_2NH), 3255 (CONH), 1640 (C=O), 1330, 1122 (SO_2). ^1H (DMSO-d_6) δ: 2.22 (s, 3H), 2.64 (t, J = 7.2 Hz, 2H), 3.32 (t, J = 7.0 Hz, 2H), 6.34 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H, Ar-H), 7.28 (d, J= 8.4 Hz, 2H, Ar-H), 7.58 (d, J = 8.4 Hz, 2H, Ar-H), 7.64 (d, J = 8.0 Hz, 2H, Ar-H), 7.78 (s, 1H, Ar-H), 8.62 (d, J = 8.2 Hz, 2H, Ar-H), 10.38 (s, 1H, SO_2NH), 11.25 (brs, 1H, CONH). ^{13}C NMR (DMSO-d_6)

δ: 26.2, 34.4, 36.7, 100.3, 103.2, 108.4, 118.2, 120.3 (2C), 123.5 (2C), 125.4 (2C), 128.7, 120.4 (2C), 132.3, 134.4, 136.2, 140.8, 142.4, 144.5, 152.7, 155.3, 164.5, 172.3, 174.2. MS: 621 (M+H).

4.3.5. 3-(4-(3-trifluoromethyl)-5-p-tolyl-1H-pyrazole-1-yl)phenylsulfonylamino)-N-(6-nitrobenzo[d]thiazol-2-yl) propanamide (4d): Yellow solid, yield: 96%; mp 251-252; IR (cm^{-1}): 3220 (SO_2NH), 3245 (CONH), 1645 (C=O), 1545, 1355 (NO_2), 1338, 1145 (SO_2). ^1H (DMSO-d_6) δ: 2.25 (s, 3H), 2.75 (t, *J* = 4.8 Hz, 2H), 4.48 (t, *J* = 5.6 Hz, 2H), 6.50 (s, 1H), 7.24 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.45 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.78 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.14 (d, *J* = 8.6 Hz, 2H, Ar-H), 8.38 (d, *J* = 8.6 Hz, 1H, Ar-H) 8.52 (s, 1H, Ar-H), 8.84 (d, *J* = 8.0 Hz, 1H, Ar-H), 10.26 (s, 1H, SO_2NH), 10.96 (brs, 1H, CONH). ^{13}C NMR (DMSO-d_6) δ: 26.5, 34.8, 36.3, 108.4, 117.3, 118.7, 121.5 (2C), 124.3, 125.7, 126.8 (2C), 127.3 (2C), 128.2, 128.7 129.5, 132.2, 135.5, 137.2, 141.3, 143.8, 145.6, 148.3, 155.2, 171.8, 173.4. MS: 630 (M+H); Anal. Calcd for $C_{27}H_{21}F_3N_6O_5S_2$: C, 51.88; H, 3.77; N, 13.76. Found C, 51.22; H, 3.43; N, 13.24.

4.3.6. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-1-(4-(4-nitrophenyl)piperazin -1-yl)propan-1-one (4e): Yellow solid, yield: 55%; mp 264-265; IR (cm^{-1}): 3242 (SO_2NH), 1631 (C=O), 1525, 1315 (NO_2), 1345, 1150 (SO_2). ^1H (DMSO-d_6) δ: 2.15 (s, 3H), 2.42 (t, *J* = 4.4 Hz, 2H), 4.25-4.52 (m, 10H), 6.45 (s, 1H), 6.28 (d, *J* = 6.6 Hz, 2H, Ar-H), 7.12 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.42 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.60 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.13 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.32 (d, *J* = 9.2 Hz, 2H, Ar-H), 10.35 (s, 1H, SO 2NH). ^{13}C NMR (DMSO-d_6) δ: 25.8, 33.4, 35.7, 44.3 (2C), 47.5 (2C), 108.3, 117.4 (2C), 118.7 (2C), 121.4 (2C), 124.2 (2C), 126.8 (2C), 127.7, 129.2 (2C), 131.7, 135.6, 136.3, 138.6, 141.2, 144.7, 153.8, 155.5, 172.5. MS: 642 (M+H); Anal. Calcd for $C_{30}H_{29}F_3N_6O_5S$: C, 56.68; H, 4.77; N, 13.76. Found C, 56.22; H, 4.13; N, 13.24.

4.3.7. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-1-(4-(4-fluorophenyl)piperazin- 1-yl)propan-1-one (4f): White solid, yield: 86%; mp 230-231; IR (cm^{-1}): 3240 (SO_2NH), 1625 (C=O), 1342, 1154 (SO_2). 1H (DMSO-d_6) δ: 2.12 (s, 3H), 2.44 (t, *J* = 4.0 Hz, 2H), 4.28-4.50 (m, 10H), 6.32 (s, 1H), 6.52 (d, J = 6.4 Hz, 2H, Ar-H), 6.84 (d, J = 7.4 Hz, 2H, Ar-H), 7.15 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.38 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.56 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.18 (d, *J* = 9.4 Hz, 2H, Ar-H), 10.50 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 26.5, 34.5, 37.2, 45.4 (2C), 47.4 (2C), 108.3, 113.4 (2C), 116.5 (2C), 120.7 (2C), 122.6 (2C), 125.4 (2C), 127.2, 130.8, 131.5, 134.3, 136.4, 138.5, 143.5, 146.3 (2C), 149.5, 155.8, 172.7. MS: 615 (M+H); Anal. Calcd for $C_{30}H_{29}F_4N_5O_3S$: C, 58.68; H, 4.72; N, 11.66. Found C, 58.12; H, 4.03; N, 11.14.

4.3.8. 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-1-(4-(2-pyrimidyl)piperazin-1-yl)propan-1-one (4g): White solid, yield: 88%; mp 234-235; IR (cm^{-1}): 3245 (SO_2NH), 1618 (C=O), 1338, 1132 (SO_2). ^1H (DMSO-d_6) δ: 2.22 (s, 3H), 2.64 (t, *J* = 3.8 Hz, 2H), 4.16-4.42 (m, 10H), 6.34 (s, 1H), 7.18 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.28 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.74 (d, *J* = 7.0 Hz, 2H, Ar-H), 8.52 (d, *J* = 8.5 Hz, 2H, Ar-H), 8.88 (s, 1H, Ar-H), 10.40 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 25.8, 34.8, 37.4, 47.2, 53.4, 108.7, 112.4, 118.5 (2C), 123.4 (2C), 125.3 (2C), 127.2, 128.4 (2C), 131.6 (2C), 133.7, 137.5, 140.3, 142.7, 145.5, 147.7, 155.4 (2C), 168.2, 173.4. MS: 599 (M+H).

4.3.9 3-(4-(3-(trifluoromethyl)-5-p-tolyl-1H-pyrazol-1-yl)phenylsulfonylamino)-1-(4-(2-pyridyl)piperazin-1-yl) propan-1-one (4h): White solid, yield: 84%; mp 238-239; IR (cm^{-1}): 3232 (SO_2NH), 1616 (C=O), 1348, 1134 (SO 2). ^1H (DMSO-d_6) δ: 2.22 (s, 3H), 2.64 (t, *J* = 4.4 Hz, 2H), 4.20-4.48 (m, 10H), 6.22 (s, 1H), 7.25 (d, *J* = 6.0 Hz, 2H, Ar-H), 7.34 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.42 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.85 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.48-8.65 (m, 3H, Ar-H), 8.92 (d, *J* = 9.0 Hz, 1H, Ar-H), 10.28 (s, 1H, SO 2NH). ^{13}C NMR (DMSO-d_6) δ: 25.3, 34.2, 37.2, 47.5, 53.4, 108.7, 113.8, 116.5, 122.7 (2C), 125.8 (2C), 127.7, 129.6 (2C), 131.3 (2C), 134.7, 137.4 (2C), 140.5 (2C), 143.7, 145.4 (2C), 147.5, 157.4, 165.4, 172.5. MS: 598 (M+H).

4.4. Antimicrobial activity

Bacterial cultures were prepared on 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. Anti-microbial tests were done by disc diffusion technique[32,33]. 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 37^0C for 24 hours. Chloramphenicol was used as positive control and was placed in the center of all the plates for bacterial cultures and nystatin was used as positive control for fungal cultures. 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.

4.5. Minimum inhibitory concentration

Minimum inhibitory concentration was evaluated using micro-broth dilution assay method[34]. MIC was determined by taking the minimum concentration at which there were observed no visually detectable bacterial/ fungal growth. Specifically, 0.1 mL of standardized inoculum ($1.2x\ 10^7$ c.f. u/ mL) was added to each test tube. The tubes were incubated aerobically at 37°C for 24 h for bacterial activity and 48-72 h for fungal activity. Control was maintained for each test sample. The lowest concentration (highest) of test compound that produced no visible signs of microbial growth (no turbidity) when compared with the control tubes were regarded as MICs (**Tables 1** and **2**).

4.6. Antioxidant activity

The radical scavenging activity of the newly synthesized compounds was measured by DPPH method[35] and Superoxide radical scavenging method[36,37] at two different concentrations 50 and 100 µg/mL. Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm.

4.7. DPPH radical scavenging activity

To 5 mL of 0.004% w/v methanol solution of DPPH, 2 mL of different concentrations of the test samples (50 and 100 µg/mL) in methanol were added. The test tubes were incubated at room temperature for 30 minutes. The solution was rapidly mixed and scavenging capacity was measured spectrometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as a standard to compare the activity of the test samples. The tests were carried out in triplicate and mean of the values were summarized in Table 3. The % inhibition of free radical production from DPPH was evaluated by using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = [(1-a)/b] \times 100$$

a= Absorbance of sample at 517 nm, b= Absorbance of control 517 nm.

4.8. Super oxide radical Scavenging activity

Super oxide radicals were determined using spectrophotometric measurement of the effects of various concentrations of test compounds on the reduction of nitrobluetetrazolium (NBT). Super oxide radicals were generated in a non-enzymatic phenazinemethosulfate/ nicotinamide adenine dinucleotide (PMS/NADH) system. The non-enzymatic generation of super oxide radicals was measured in reaction mixtures containing various concentrations of test compounds (50 and 100 µg/mL), PMS (15 µM), NADH (73 µM) and NBT (50 µM) in phosphate buffer (20 mM, p H 7.4). After incubation for 5 minutes at ambient temperature, the color was read at 560 nm against blank samples.

Super oxide radical Scavenging activity (%) = [(1-a)/b] x 100
a= Absorbance of control, b= Absorbance of test samples

Reactive oxygen species (ROS), such as superoxide anion radical (O^{2-}), hydroxy radical ($OH^{\cdot}$) and peroxy radicals ($ROO^{\cdot}$) are produced as a part of normal metabolic processes. The compounds **4c, 4f** and **4e** showed significant antioxidant activity by scavenging the free radicals and super oxide radicals due to the presence of high electronegative halogens (Cl, F) and NO_2 group. The antioxidant activity evaluation with the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), radical scavenging assay was carried out and the results are presented in **Table 3.**

5. Conclusion

In conclusion, we have described the design and synthesis of new carboxamide derivatives of celecoxib by structural modification at sulfonamide group and tested for their antimicrobial and antioxidant activities. Summarizing the biological results, it can be seen that the pharmacological efficacy of the synthesized compounds showed good inhibitory activity. Particularly the compounds **4c-f** displayed pronounced activity against tested strains. From the antioxidant and antimicrobial activity results, it can be concluded that, a combination of two different heterocyclic motifs namely pyrazoles, benzothiazole, piperazine and pyrimidine exhibited augmented biological properties. Hence, drug candidates like celecoxib having bioisosters are ideally apt for the further modifications which will contribute varied pharmacological activities.

Acknowledgments

The authors thank to S V University, Tirupati for providing the lab facilities and IICT, Hyderabad for recording the spectra.

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