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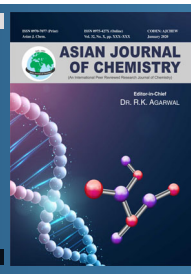

Synthesis, Molecular Docking, DFT Study of Novel *N*-Benzyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxamide Derivatives and their Antibacterial Activity

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A series of febuxostat based new chemical entities was synthesized using microwave method and characterized by NMR, mass and FT-IR spectral studies. Molecular docking of febuxostat amide nucleus substitution compounds **8c** (-7.91kcal/mol), **8g** (-7.94 kcal/mol) exhibiting high binding energy against ALK receptors. Theoretical investigation of MEPs, HOMO, LUMO and energy gap of HOMO-LUMO were calculated by B3LYP/6-31G method. Among the tested compounds, methoxy substituted compound **8g** showed highest antibacterial activity against *S. aereus* and *B. subtilis*.

Keywords: Microwave method, Febuxostat amides, Antibacterial activity, DFT study.

INTRODUCTION

Febuxostat is a drug existing in the market and recently used to clinically treat hyperuricemia [1], which is used as a xanthine oxidase inhibitor. Febuxostat has been shown to effectively reduce serum urate level in preclinical and clinical studies [2-6]. Febuxostat received approval in 2009 from the U.S. Food and Drug Administration for chronic management of hyperuricemia in patients with gout and cancer [7]. The great significance of xanthine oxidase has been clinically used to treat vascular diseases, liver damage and chronic heart failure [8].

Thiazole heterocycle is a significant bioactive unit in many drugs. A number of thiazole containing drugs have been demonstrated as very good antibacterial agents. Thiazole plays vital roles in many drug structures and having varied biological activity such as antifungal [9], hypertension [10], antibacterial [11,12], HIV infections [13], antimycobacterial [14], hypnotics [15], antipsychotic [16], anti-inflammatory [17], anticancer [18], *etc*. Herein, we describe the synthesis of thiazole derivatives containing an amide skeleton as antibacterial agents [19, 20]. Tyrosine kinase anaplastic lymphoma kinase (ALK) is a validated tyrosine kinase target in several cancers, including non-small-cell lung cancer, anaplastic large-cell lymphoma, and pediatric neuroblastoma [21-23]. ALK rearrangements are found in approximately 5 % of cases of non-small-cell lung cancer and define a distinct molecular subtype of lung cancer [24-27]. With an estimated 1.3 million new cases of non-small-cell lung cancer worldwide each year [28]. Lung cancer expresses the most important causes of cancer-related mortality [29]. Lung cancers through ALK rearrangements are highly responsive to ALK tyrosine kinase inhibition, underscoring the notion that such cancers are addicted to ALK kinase activity. Based on early phase studies, the multi-targeted tyrosine kinase inhibitor (TKI) crizotinib was approved by the FDA to treat patients with advanced NSCLC harboring ALK rearrangements [21,30]. However, despite a high response rate of 60 % in ALK-rearranged NSCLC, most patients develop resistance to crizotinib, typically within one to two years. Thus, crizotinib became the first new drug approved for lung cancer in the past six years [31]. Given its excellent activity, an editor of the New England Journal of Medicine praised crizotinib as a new champion in the cancer war [32]. None of the reports available for the synthesis of febuxostat/febuxostat derivatives by microwave approach. On the basis of this knowledge, the research is decided to synthesize febuxostat amides by microwave approach.

EXPERIMENTAL

All chemicals used for the synthesis of the desired compounds were obtained from Loba Chime, Spectrochem, India and Merck. The FT-IR analysis of the synthesized compounds were recorded in FTIR 8300, KBr press, Shimadzu. Mass studies of the synthesized compounds were performed by using the instrument SHIMADZU QP 500. ^{1}H NMR spectra were acquired on a Bruker 300 MHz spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as an internal standard. The microwave reactions were carried out in a biotage microwave synthesizer.

Biological assay: The antibacterial activities of all test compounds were carried out by disc diffusion method. The concentrations of the test compounds were fixed at 100 and 200 µg. The standard drug is used as streptomycin. The target microorganisms were cultured in Muller-Hinton broth (MHB). After 24 h, the suspensions were adjusted to standard sub culture dilution. The petri-dishes containing Muller Hinton agar (MHA) medium were cultured with diluted bacterial strain. Disc made of Whatman no.1, diameter 6 mm was presterilized and was maintained in the aseptic chamber. Each concentration was injected into the sterile disc papers. Then, prepared discs were placed on the culture medium. Standard drug streptomycin (10 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antibacterial activity.

General procedure for the synthesis of febuxostat amide derivatives (8a-j): Compound **7** (0.2 g, 0.632 mmol) was dissolved in acetonitrile (2 mL). To that solution, TBTU (0.243 g, 0.757 mmol) and triethylamine (0.132 mL, 0.948 mmol) was added and stirred for 30 min under nitrogen atmosphere. The amine (0.632 mmol) was added and stirred for 3 h at room temperature. The completion of the reaction was monitored by TLC and extracted with ethyl acetate. The organic layer was washed with sodium bicarbonate solution, water, brine solution, which was separated and dried over anhydrous sodium sulphate. The evaporation of solvent yielded target compounds (yield: 85 %) (**Scheme-I**).

Spectral data

***N*-Benzyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxamide (8a):** ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.09-8.12 (2H, m, Ar-H); 7.27-7.36 (5H, m, Ar-H); 7.00-7.02 (1H, d, Ar-H); 4.62 (2H, d, -CH_2); 3.88-3.90 (2H, d, -CH_2); 2.74 (3H, s, -CH_3); 2.24-2.25 (1H, m, -CH); 1.07-1.10 (6H, d, -CH_3). FT-IR (KBr, ν_{max}, cm^{-1}): 2965.98 (-CH), 2222.56 (-C≡N), 1636.3 (-C=O), 1527.35 (-C=N), 1174.44 (-C=C). ESI-MS: Calculated 405.51, Found 406.2 (M+1)$^+$. Anal. calcd. (%) for $C_{23}H_{23}N_3O_2S$: C, 68.12; H, 5.72; N, 10.36; S, 7.91; Found (%): C, 68.14; H, 5.71; N, 10.35; S, 7.90.

2-(3-Cyano-4-isobutoxyphenyl)-*N*-(4-methoxybenzyl)-4-methylthiazole-5-carboxamide (8b): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.02-8.12 (2H, m, Ar-H); 7.26-7.30 (2H, m, Ar-H); 6.98-7.01 (1H, m, Ar-H); 6.88-6.91 (1H, m, Ar-H); 6.04 (1H, m, Ar-H), 4.54-4.56 (2H, d, -CH_2); 3.87-3.90 (2H, d, -CH_2); 3.81 (3H, s, -CH_3); 2.71 (3H, s, -CH_3); 2.09-2.16 (1H, m, -CH); 1.07-1.09 (6H, d, -CH_3). FT-IR (KBr, ν_{max}, cm^{-1}): 2956.3 (-CH), 2875.3 (-C≡N), 1627.8 (-C=O), 1534.1 (-C=N), 1170.6 (-C=C). ESI-MS: Calculated 435.53, Found 436.2 (M+1)$^+$. Anal. calcd. (%) for $C_{24}H_{25}N_3O_3S$: C, 66.18; H, 5.79; N, 9.65; S, 7.36: Found (%): C, 66.18; H, 5.79; N, 9.65; S, 7.36; Found (%): C, 66.71; H, 5.77; N, 9.62; S, 7.38.

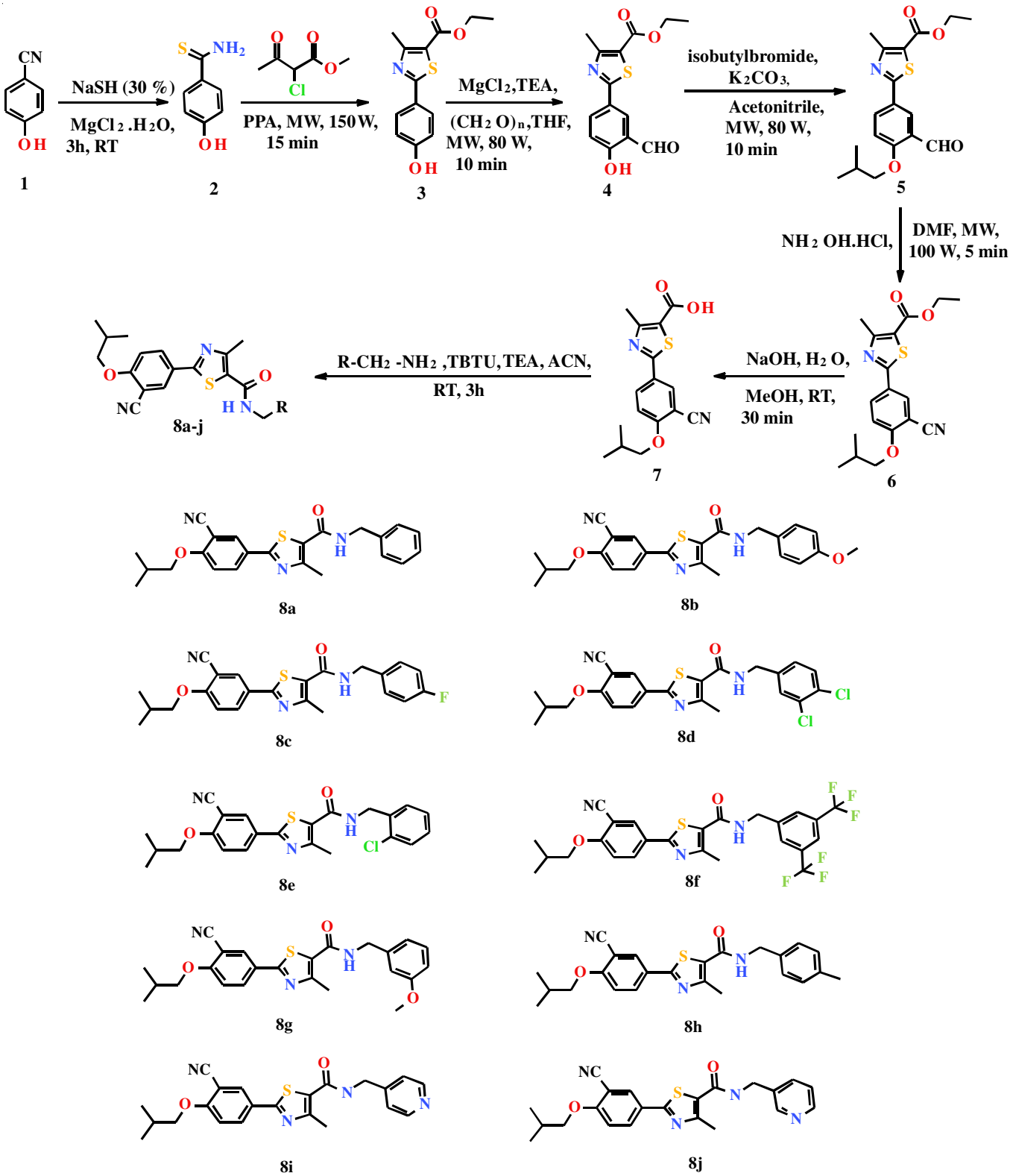
2-(3-Cyano-4-isobutoxyphenyl)-*N*-(4-fluorobenzyl)-4-methylthiazole-5-carboxamide (8c): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.02-8.12 (2H, m, Ar-H); 7.26-7.33 (2H, m, Ar-H); 6.98-7.08 (2H, m, Ar-H); 6.12 (1H, m, Ar-H), 4.57-4.59 (2H, d, -CH_2); 3.88-3.90 (2H, d, -CH_2); 2.75 (3H, s, -CH_3); 2.17-2.20 (1H, m, -CH); 1.07-1.09 (6H, d, -CH_3). FT-IR (KBr, ν_{max}, cm^{-1}): 2958.3 (-CH), 2872.5 (-C≡N), 1644.0 (-C=O), 1535.1 (-C=N), 1171.5 (-C=C). ESI-MS: Calculated 423.50, Found 424.2 (M+1)$^+$. Anal. calcd. (%) for $C_{23}H_{22}N_3O_2SF$: C, 65.23; H, 5.24; N, 9.92; S, 7.57; Found (%): C, 65.25; H, 5.22; N, 9.91; S, 7.58.

2-(3-Cyano-4-isobutoxyphenyl)-*N*-(3,4-dichlorobenzyl)-4-methylthiazole-5-carboxamide (8d): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.16-8.18 (2H, m, Ar-H); 7.39 -7.46 (2H, m, Ar-H); 7.23-7.27 (1H, d, Ar-H); 7.05 (1H, d, Ar-H); 4.56 (2H, d, -CH_2); 3.91 (2H, d, -CH_2); 2.77 (3H, s, -CH_3); 2.16-2.24 (1H, m, -CH); 1.07-1.09 (6H, d, -CH_3). FT-IR (KBr, ν_{max}, cm^{-1}): 2960.2 (-CH), 2875.34 (-C≡N), 1635.44 (-C=O), 1531.2 (-C=N), 1167.6 (-C=C). ESI-MS: Calculated 474.40, Found 474.1 (M+1)$^+$. Anal. calcd. (%) for $C_{23}H_{21}N_3O_2SCl_2$: C, 58.23; H, 4.46; N, 8.86; S, 6.76; Found (%): C, 58.24; H, 4.47; N, 8.89; S, 6.78.

***N*-(2-Chlorobenzyl)-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxamide (8e):** ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 8.81-8.83 (1H,t, -NH); 8.25-8.26 (2H, m, Ar-H); 7.46-7.47 (1H, d, Ar-H); 7.35-7.39 (4H, m, Ar-H); 4.50-4.52 (2H, d, -CH_2); 4.00-4.01 (2H, t, -CH_2); 2.62 (3H, s, -CH_3); 2.08-2.09 (1H, m, -CH); 1.01-1.02 (6H, d, -CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ ppm: 16.85, 17.05, 18.61, 22.12, 27.54, 75.08, 101.53, 113.95, 115.37, 127.14, 128.69, 128.80, 131.21, 132.82, 151.14, 155.14, 161.05, 161.81. FT-IR (KBr, ν_{max}, cm^{-1}): 2960.2 (-CH), 2873.4 (-C≡N), 1626.7 (-C=O), 1542.8 (-C=N), 1156.1 (-C=C). ESI-MS: Calculated 439.95, Found 440.1 (M+1)$^+$. Anal. calcd. (%) for $C_{23}H_{22}N_3O_2SCl$; C, 62.79; H, 5.04; N, 9.55; S, 7.29; Found (%): C, 62.81; H, 5.06; N, 9.58; S, 7.32.

***N*-(3,5-*Bis*(trifluoromethyl)benzyl)-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxamide (8f):** ^{1}H NMR (500 MHz, DMSO-d_6), δ ppm: 8.95-8.97 (1H,t, -NH); 8.25-8.26 (1H, d, Ar-H); 8.17-8.20 (1H, m, Ar-H); 8.02-8.03 (3H, d, Ar-H); 7.37-7.39 (1H, m, Ar-H); 4.62-4.63 (2H, d, -CH_2); 4.00-4.01 (2H, d, -CH_2); 2.61 (3H, s, -CH_3); 2.07-2.09 (1H, m, -CH); 1.01-1.02 (6H, d, -CH_3). ^{13}C NMR (75 MHz, DMSO-d_6), δ ppm: 16.99, 18.66, 27.54, 42.21, 75.08, 101.53, 113.91, 120.70, 125.38, 128.19, 131.24, 132.83, 142.83, 155.33, 161.25, 161.84, 163.98. FT-IR (KBr, ν_{max}, cm^{-1}): 2964.05 (-CH), 2878.24 (-C≡N), 1617.98 (-C=O), 1534.1 (-C=N), 1167.69 (-C=C). ESI-MS: Calculated 541.50, Found 543.1 (M+1)$^+$. Anal. calcd. (%) for $C_{25}H_{21}N_3O_2SF_6$; C, 55.45; H, 3.91; N, 7.76; S, 5.92; Found (%): C, 55.47; H, 3.94; N, 7.77; S, 5.95.

2-(3-Cyano-4-isobutoxyphenyl)-N-(3-methoxybenzyl)-4-methylthiazole-5-carboxamide (8g): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.20-8.28 (3H, m, Ar-H); 7.91-7.94 (1H, m,



Scheme-I: Synthesis of febuxostat amide derivatives (**8a-j**)

Ar-H); 7.61-7.66 (1H, m, Ar-H); 7.04-7.07 (1H, m, Ar-H); 7.03-6.82 (1H, m, Ar-H), 4.60 (2H, d, -CH_2); 3.90 (2H, d, -CH_2); 3.80 (3H, s, -CH_3); 2.76 (3H, s, -CH_3); 2.09-2.26 (1H, m, -CH); 1.07-1.09 (6H, d, -CH_3).). ^{13}C NMR (75 MHz, DMSO-d_6), δ ppm: 17.01, 18.61, 27.54, 42.72, 54.95, 75.07, 101.51, 112.16, 112.90, 113.93, 119.35, 125.48, 129.37, 131.18, 132.79, 140.76, 154.83, 159.28, 160.87, 163.67. FT-IR (KBr, ν_{max}, cm^{-1}): 2970.8 (-CH), 2361.41 (-C≡N), 1618.95 (-C=O), 1541.81 (-C=N), 1169.62 (-C=C). ESI-MS: Calculated 435.53, Found 436.2 $(M+1)^+$. Anal. calcd. (%) for $C_{24}H_{25}N_3O_3S$: C, 66.18; H, 5.79; N, 9.65; S, 7.36; Found (%): C, 66.21; H, 5.81; N, 9.63; S, 7.39.

2-(3-Cyano-4-isobutoxyphenyl)-4-methyl-*N*-(4-methylbenzyl)thiazole-5-carboxamide (8h): ^{1}H NMR (300 MHz,

$CDCl_3$), δ ppm: 8.13-8.14 (1H, m, Ar-H); 8.03-8.07 (1H, m, Ar-H); 7.27-7.29 (2H, m, Ar-H); 6.98-7.01 (1H, m, Ar-H); 6.87-6.90 (1H, m, Ar-H); 6.33 (1H, m, Ar-H), 4.69-4.71 (2H, d, $-CH_2$); 3.88-3.90 (2H, d, $-CH_2$); 3.72 (3H, s, $-CH_3$); 2.18-2.24 (1H, m, -CH); 1.58 (3H, s, $-CH_3$); 1.07-1.09 (6H, d, $-CH_3$). FT-IR (KBr, ν_{max}, cm^{-1}): 2964.1 (-CH), 2229.3 (-C≡N), 1606.4 (-C=O), 1532.2 (-C=N), 1168.7 (-C=C). ESI-MS: Calculated 419.53, Found 420.2 $(M+1)^+$. Anal. calcd. (%) for $C_{24}H_{25}N_3O_2S$; C, 68.71; H, 6.01; N, 10.02; S, 7.64; Found (%): C, 68.72; H, 6.03; N, 10.06; S, 7.67.

2-(3-Cyano-4-isobutoxyphenyl)-4-methyl-*N*-(pyridin-4-ylmethyl)thiazole-5-carboxamide (8i): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.12-8.13 (1H, m, Ar-H); 8.03-8.06 (1H, m, Ar-H); 7.17-7.26 (3H, m, Ar-H); 6.98-7.01 (1H, d, Ar-H); 6.02 (1H, m, Ar-H), 4.57-4.59 (2H, d, $-CH_2$); 3.88-3.90 (2H, d, $-CH_2$); 2.72 (3H, s, $-CH_3$); 2.18-2.22 (1H, m, -CH); 1.07-1.09 (6H, d, $-CH_3$). FT-IR (KBr, ν_{max}, cm^{-1}): 2928.4 (-CH), 2230.3 (-C≡N), 1608.3 (-C=O), 1531.2 (-C=N), 1164.8 (-C=C). ESI-MS: Calculated 406.50, Found 404.2 $(M-1)^+$. Anal. calcd. (%) for $C_{22}H_{22}N_4O_2S$; C, 65.00; H, 5.46; N, 13.78; S, 7.89; Found (%): C, 65.03; H, 5.45; N, 13.80; S, 7.93.

2-(3-Cyano-4-isobutoxyphenyl)-4-methyl-*N*-(pyridin-3-ylmethyl)thiazole-5-carboxamide (8j): ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 8.12-8.13 (1H, m, Ar-H); 8.03-8.06 (1H, m, Ar-H); 7.17-7.26 (3H, m, Ar-H); 6.98-7.01 (1H, d, Ar-H); 6.01 (1H, m, Ar-H), 4.57-4.59 (2H, d, $-CH_2$); 3.88-3.90 (2H, d, $-CH_2$); 2.72 (3H, s, $-CH_3$); 2.18-2.22 (1H, m, -CH); 1.07-1.09 (6H, d, $-CH_3$). FT-IR (KBr, ν_{max}, cm^{-1}): 2954.4 (-CH), 2927.4 (-C≡N), 1628.6 (-C=O), 1531.2 (-C=N), 1165.8 (-C=C). ESI-MS: Calculated 406.50, Found 404.2 $(M-1)^+$. Anal. calcd. (%) for $C_{22}H_{22}N_4O_2S$; C, 65.00; H, 5.46; N, 13.78; S, 7.89; Found (%): C, 65.04; H, 5.48; N, 13.76; S, 7.90.

RESULTS AND DISCUSSION

The target molecules (**8a-j**) were synthesized from 4-hydroxybenzonitrile by using microwave approach (**Scheme-I**). The reaction of precursor **1** with sodium hydrogen sulfide in the presence of magnesium chloride monohydrate provided 4-hydroxybenzothioamide (**2**). The cyclization of a thioamide derivative with methyl 2-chloro-3-oxobutanoate yielded thiazole intermediate **3**. Then intermediate **5** was synthesized from the intermediate **3** *via* intermediate **4** by regular formylation and alkylation reactions. The aldehyde was converted to nitrile with the use of hydroxylamine hydrochloride at reflux condition. The hydrolysis of ester yielded the major intermediate **7**. Finally, febuxostat amides were synthesized from the intermediate **7** with the use of TBTU as the coupling agent.

Most of the intermediates were synthesized by microwave approach. These reactions gave improved yield than conventional heating methods. The reactions likewise completed in few minutes. The synthesized compounds were characterized by ^{1}H NMR, mass and FT-IR spectroscopies. The disappearance of carboxylic acid proton and the appearance of amide proton in ^{1}H NMR clearly indicate the amide formation. The appearance of singlet for 2 protons at 4.6 ppm, doublet for 2 protons at 3.8 ppm indicates the presence of benzyl CH_2 unit and isobutyl CH_2 units. The methyl protons present in a thiazole ring appeared at 2.7 ppm. Similarly, isobutyl units (CH_3 and CH) appeared at 2.4 ppm (1H, m) and 1.0 ppm (6H, d). The aromatic protons appeared at their corresponding regions. The Electrospray ionization mass spectrum clearly displayed parent peak in the positive mode region. The FT-IR results contributed some additional information for functional groups like amide, ether and nitrile.

Molecular docking methodology was used to identify the structural features required for ALK receptor [PDB ID: 2XP2 (crizotinib)]. The docking study of febuxostat amides with receptor ALK exhibited good binding with one or more amino acids in the receptor active pocket. Compounds **8a**, **8c**, **8d**, and **8g** showed very high binding energy with the ALK (2XP2) receptor (Fig. 1). Compound **8a** exhibits binding energy value -7.90 kcal/mol showed two H-bonding with Ala1200 as well as the strong affinity of Leu1122, Val1130, Leu1256 which results from six hydrophobic interaction its reduced the hydrogen bond interaction of compound **8a** with ALK receptor. The electron withdrawing fluorine atom of compound **8c** also exhibits strong binding energy -7.91 kcal/mol, which result two H-bonding with Tyr1211, Glu1210 and as well as Pro1260, Leu1122 and Phe1207 amino acids, which results hydrophobic and electrostatic interactions to responsible for more activity of ALK receptor and its indicated that the presence of febuxostat nucleus has a higher affinity for ALK protein.

The electron withdrawing group of compound **8d** shows binding energy -7.27 kcal/mol was deeply embedded in the hydrophobic pocket formed by Met1199, Leu1198, Leu1196, Ala1148, Leu1122, Val1130, Leu1256 exhibits hydrophobic bond with a chlorine atom, including Arg1253, Pro1292 binding with febuxostat and benzene nucleus, which result shows highest antibacterial activity. Moreover, compound **8g** exhibits highest binding energy value -9.64 kcal/mol interact with two hydrogen bonded amino acid residue Tyr1278, Ile1277 and inside of strong hydrophobic interaction with Arg1279, Phe1098 and His1244 amino acid residue, due to the presence of electron donating group in phenyl ring at *meta*-position. The free energy of binding (FEB) values of all compounds was calculated are reported in Table-1.

TABLE-1
DOCKING RESULTS OF NOVEL BIOLOGICAL ACTIVE FEBUXOSTAT AMIDES DERIVATIVES AGAINST ALK [2x2p.pdb] PROTEIN

Compounds	Binding energy (kcal/mol)	Number of hydrogen bonding
8a	-7.90	1
8b	-6.72	3
8c	-7.91	2
8d	-7.27	1
8e	-7.12	1
8f	-5.56	2
8g	-7.94	2
8h	-6.32	1
8i	-6.81	2
8j	-6.71	1

DFT studies: The calculated gap value in gaseous phase shows the highest chemical reactivity due to the lowest energy gap value signifying easy molecular charge transfers and hence binding with a receptor. HOMO and LUMO and their gap were

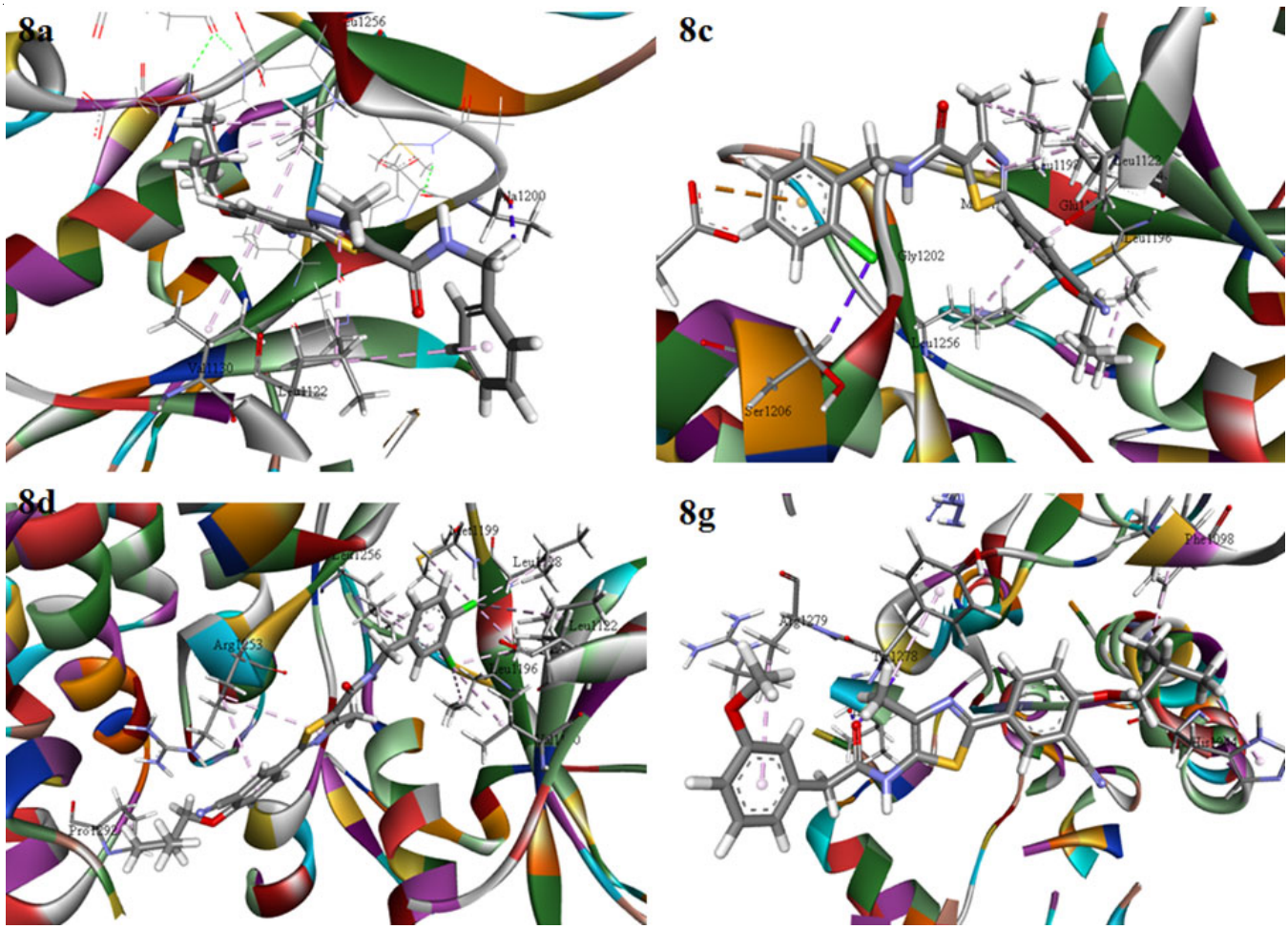


Fig. 1. Binding mode of the most active compound **8a**, **8c**, **8d**, **8g** with ALK receptor, The amino acids involved in hydrogen (blue dashed line), hydrophobic (white dashed line) and electrostatic (red dashed line) interactions are highlighted

calculated for all the compounds and are reported in Table-2. It is significant that compounds **8a**, **8c** and **8g** having the lowest energy gap (ΔE) of 4.2591, 4.1720 and 4.1862 eV, respectively, show the highest biological activity [33-35]. $E_{HOMO} - E_{LUMO}$ band gap values in compound **8g** was lower than another molecule for the gas phase, which could be due to the electron attracting methoxy group attached to phenyl ring of compound **8g**.

The plots of HOMO and LUMO of some molecules obtained from DFT calculations are displayed in Fig. 2. The results illustrated that HOMO and LUMO molecular orbital of compounds **8a** and **8c** was mainly located in febuxostat amide with aromatic ring indicating the existence of possible reactive sites; consequently, electrophilic attacks might take place on these sites. The HOMO lobe compound **8g** was primarily located in methoxy substituted phenyl ring comparing with other compounds. The LUMO of compound **8g** presents similar characteristic, while the HOMO changes significantly compared to other derivatives.

Molecular electrostatic potential surface (MEPs): Molecular electrostatic potential is related to the electronic density which is a very useful descriptor in understanding sites for nucleophilic reactions or electrophilic attack. As a result, this property was calculated for the target molecules at B3LYP/6-31G level of theory and indicated by a colour range from

TABLE-2
ENERGIES OF BOTH HOMO AND LUMO AND THEIR GAPS (eV) CALCULATED FOR ALL SYNTHESIZED COMPOUNDS (**8a-j**)

Compounds	$E_{total\ (kcal)}$ E_{HF}	μ (Debye)	E_{HOMO}	E_{LUMO}	ΔE
8a	-1603.1097	8.3407	6.3859	2.1268	4.2591
8b	-1717.5894	9.0201	6.4406	2.0321	4.4085
8c	-1702.2994	7.0714	6.5742	2.4022	4.1720
8d	-2522.2183	5.1333	6.8542	2.4560	4.3982
8e	-2062.6174	5.7555	6.7535	2.3429	4.4106
8f	-2277.1309	5.6144	6.9008	2.5848	4.3160
8g	-1717.5284	6.7176	6.5383	2.3521	4.1862
8h	-1642.3243	7.7213	6.6553	2.2256	4.4297
8i	-1619.0480	6.9967	6.7595	2.4618	4.2977
8j	-1619.0490	6.1994	6.7538	2.3608	4.3930

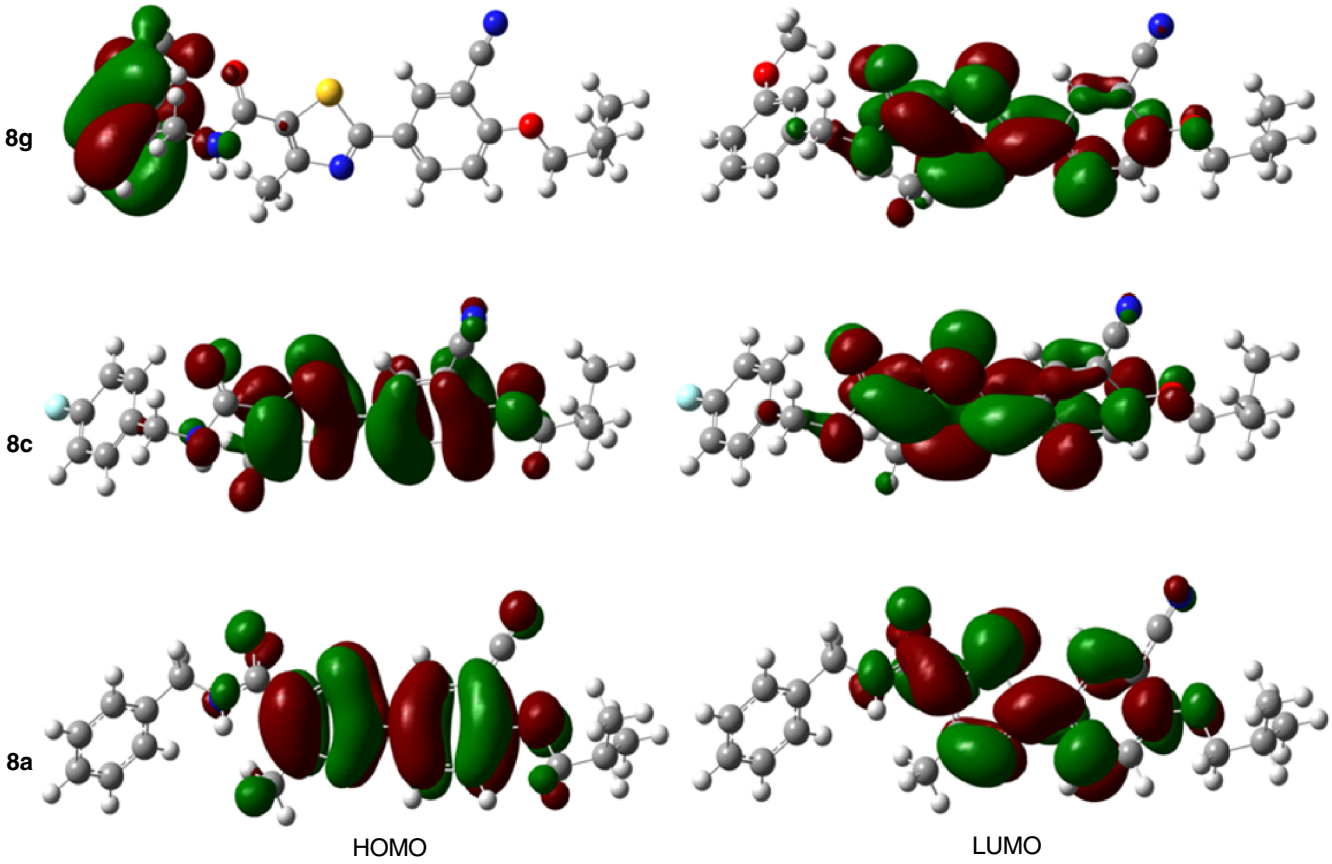


Fig. 2. Plots of the HOMO and LUMO density map of most active febuxostat amide compounds

-3.1620×10^{-2} (deep red) to $+3.1620 \times 10^{-2}$ (deep blue) in the corresponding maps displayed. MEP was created by mapping of the electrostatic potential on the total electron density of the molecules. For most active compounds **8a**, **8c** and **8g** shows in red the nucleophilic sites (negative potential), located at the oxygen atoms which result in H-bond interaction with the amino acid of target receptor. The large electrophilic sites (positive potential) appeared on the hydrogen attached to aromatic ring nitrogen consequence the blue cloud which was symbolized for electron deficient region. Due to the accumulation of positive potential, these moieties exhibited hydrophobic interactions with the aromatic residues of active site in Fig. 3. The other parts of a molecule seem to potentially be less active in chemical point of view.

Antibacterial activity: All the synthesized compounds were screened for their antibacterial activity by disc diffusion technique. The febuxostat amides were screened for their *in vitro* antimicrobial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-9144), *Klebsiella pneumonia* (ATCC-13883) and *Bacillus subtilis* (ATCC-6051) were compared with standard drug streptomycin and the zones of inhibition were calculated. Among the synthesized compounds, compounds **8b**, **8c**, **8d**, **8f** and **8j** containing 4-methoxybenzyl, 4-fluorobenzyl, 3,4-dichlorbenzyl, 3,5-di(trifluoromethy)-benzyl and 3-aminomethyl pyridine substituents demonstrated inhibition against all the pathogens. Compounds **8a** and **8i** having benzyl and 4-aminomethyl pyridine substituents exhibited activity against *S. aureus*, *B. subtilis* and *K. pneumonia*. Compound **8g** having 3-methoxybenzyl substituents showed

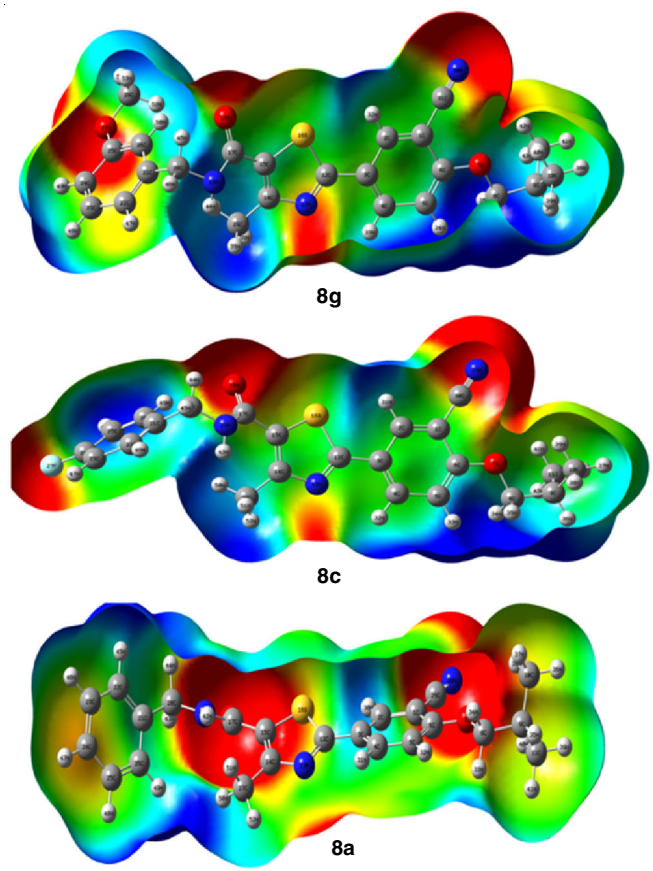


Fig. 3. Electrostatic potential mapping on the electron density of compounds **8a, 8c** and **8g**

TABLE-3
ANTIBACTERIAL ACTIVITY OF FEBUXOSTAT AMIDE DERIVATIVES

Compound	Zone of inhibition (mm)							
	S. aereus		*B. subtilis*		*E. coli*		*K. pneumonia*	
	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
8a	8	13	7	13	15	9	8	14
8b	9	14	9	14	11	13	10	14
8c	8	12	9	13	10	14	9	13
8d	9	15	8	14	11	15	8	11
8e	–	8	7	9	–	–	–	–
8f	–	13	–	10	–	8	–	10
8g	14	16	11	15	10	13	9	15
8h	–	–	–	–	–	–	7	10
8i	7	10	7	9	–	–	7	9
8j	8	12	8	12	9	12	8	12
Standard: Streptomycin	24		25		24		23	
Control: DMSO	–		–		–		–	

an activity against only Gram-negative pathogens. Also, compound **8h** containing 4-methyl benzyl substituents showed an inhibition against *K. pneumonia* only. Amid the synthesized compounds, compound **8a**, **8c**, **8d** and **8g** gave good response against pathogens. The zone of inhibition (in mm) of the microorganisms is represented in Table-3.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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