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Synthesis, HOMO-LUMO Analysis and Antioxidant Activity of Novel Tetrazole Hybrids

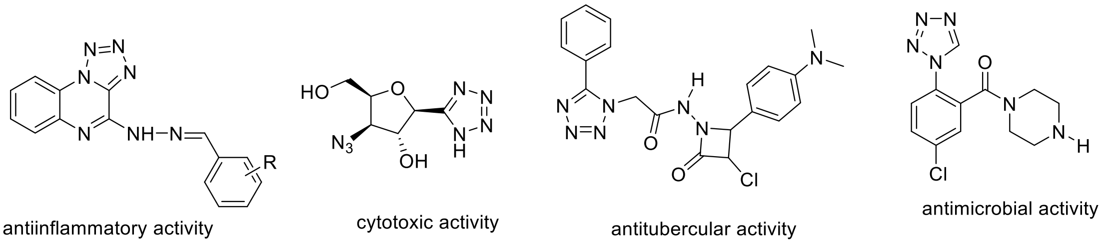
In this work a series of twelve tetrazole hybrids **7a-l** were prepared *via* Cu promoted regioselective *N*-arylation, C-N bond formation reactions and assed for *in vitro* anti-oxidant activity. Spectroscopic methods like ^{1}H NMR, ^{13}C NMR, IR, and LCMS were used for the characterization of the obtained compounds and all data were in good agreement with the expected structures. Next, to study the chemical reactivity, the HOMO-LUMO energies and their energy gap, hardness, softness, chemical potential of the synthesized chemical compounds **7a-j** were calculated by B3LYP/6-31g(d, p) method using Gaussian 09 package. Compounds **7d, 7e, 7j** have demonstrated the lowest HOMO-LUMO energy gap (4.7856eV, 4.7905eV, 4.89009eV), and the lowest chemical potential (-3.07254eV, -3.06173eV, -3.0166eV) values, also the highest chemical softness (0.41792eV, 0.41749eV,0.40899eV), and the lowest chemical hardness (2.3928eV, 2.3952eV, 2.44504eV) values, which may correspond to higher chemical reactivity compared with other compounds of this series. Moreover, obtained compounds **7a-l** were screened *in vitro* for the antioxidant activity by DPPH radical scavenging method. The screening results revealed that compounds **7d, 7e** and **7k** are the most potent among all the examined compounds, and the remaining compounds have moderate activity.

Keywords: Tetrazole, Morpholine, Cu catalyst, Regio-selectivity, anti-oxidant activity, DPPH, Gaussian 09, DFT, HOMO-LUMO analysis.

Introduction

Nitrogen-heterocyclic scaffolds are one of the most common structural motifs in pharmaceuticals [1]. Tetrazoles contain the highest amount of nitrogen among the stable heterocyclic scaffolds. They do not exist in nature and are one of the important cores of aromatic heterocyclic systems, which have wide application in various fields [2–4]. However, the most advantageous and fruitful use of tetrazoles is in medicinal chemistry [5–7]. Furthermore, tetrazoles are also used as lipophilic spacers [8], peptide chelating agents [9], as *cis*-peptide bond mimics [10]. Due to the tunable lipophilicity, tetrazole derivatives have been employed as "isosteres" of several functional groups, such as carboxylic acid moiety [11]. Amino tetrazole is found in compounds having anti-allergic/anti-asthmatic [12], antiviral and anti-inflammatory [13], anti-neoplastic [14] activities. Substituted 5-aminotetrazoles exhibit versatile biological activities such as antidiabetic [15] and antibiotic [16], acts as excellent anti-corrosion agent of stainless steel [17] and are used as cholecystokinin B (CCK-B) receptor antagonists [18] and as ligands in coordination chemistry [19]. Recent studies have described the use of 5-aminotetrazoles as photoprecursors of reactive intermediates [20].

Moreover, the tetrazole function is metabolically stable and has a close similarity between the acidic character of the tetrazole group and the carboxylic acid group, which has inspired the medicinal chemists to synthesize substituted tetrazoles as potential medicinal agents (Fig. 1).



antiinflammatory activity

cytotoxic activity

antitubercular activity

antimicrobial activity

Figure 1. Some of the bioactive compounds with tetrazole skeleton

Morpholine, is an attractive pharmacophore substance, its presence in a molecule leads to various biological actions [21–23]. The presence of fluorine can lead to significant changes in their physical, chemical, and biological properties, thereby improving bioavailability [24]. In medicinal chemistry, molecular hybridization for drug development is one of the advanced techniques; combining pharmacophoric units of different bioactive substances creates a hybrid with greater affinity and potency than the standard drug [25, 26].

Free radicals such as nitric oxide, superoxide, and hydroxyl are oxygen based free radicals, and are also called reactive oxygen species (ROS). ROS are formed in the human body and would damage proteins, lipids and DNA, leading to a variety of diseases like inflammation, carcinogenesis, and drug-associated toxicity. Moreover, radical reactions play an important role in the progress of life-limiting chronic diseases such as diabetes, cancer, arteriosclerosis, ageing, and others [27]. Antioxidants are synthetic or natural molecules that can interact with free radicals and prevent their chain reactions before vital molecules are damaged [28]. Hence, they have recently recognized as drug candidates to combat these assorted diseases such as inflammation, atherogenesis, carcinogenesis and aging in aerobic organisms [29, 30]. Accordingly, the development of new compounds with potent antioxidant properties is becoming one of the most momentous area of medicinal chemistry research.

Given these results, it was considered interesting to embark on the synthesis of 5-aminotetrazole derivatives with morpholine, suggesting that these compounds might possess certain antioxidant activity. Based on these reports and in continuation of our efforts towards the development of viable heterocyclic scaffolds with therapeutic value [31–33], in this paper we presented the synthesis of some morpholine based tetrazole hybrids and evaluated their antioxidant properties.

Experimental

General Information: All the chemicals were purchased from Merck, SRL-India, Finar and used without further purification. Melting points were determined by the open glass capillary technique [34] on a Cintex melting point equipment. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum one FT-IR spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer using $CDCl_3$ and DMSO as a solvent. All the reactions were monitored by TLC on pre-coated silica gel plates (60F 254; Merck). Column chromatography was performed on 100–200 mesh silica gel (SRL, India). VKSI Medico Centrifuge machine was used for our experimental procedure for the synthesis of resulting compounds.

*Experimental procedure for the synthesis of 1-(3,4-difluorophenyl)-1H-tetrazol-5-amine (**3**):*

To a stirred solution of DMSO (2–3 ml), 5-amino tetrazole (**1**) (1 mmol, 85 mg), 1,2-difluoro-4-iodobenzene (1 mmol, 240 mg) were slowly added. Then Cs_2CO_3 (1 mmol, 325 mg) and $Cu(OAc)_2 \cdot H_2O$ (10 mol %, 20 mg), 1,10-phenanthroline (20 mol %, 36 mg) were added consecutively for several min and the reaction mixture was stirred for 18 h at 85 °C. The reaction progress was monitored by TLC (5 % ethylacetate in hexane). After completion of the reaction (monitored by TLC), the reaction mixture was transferred into centrifuge tubes and centrifuged for 10 min using centrifugation machine. A black solid was settled at the bottom of the centrifuged tubes. The transparent solution was concentrated by using rotary evaporator and the crude mixture was purified by silica gel (60–120 mesh) column chromatography using 30 % ethylacetate in hexane as eluent to obtain a phenyl tetrazole amine to afford 1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine (**3**) as pale yellowish solid.

*Procedure for the synthesis of 1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine (**5**):*

In DMSO (5 mL), 1-(3,4-difluorophenyl)-1H-tetrazol-5-amine **3**, (31.5 mmol, 6.2 g) was added and stirred for 10 min. Next, to this stirred solution Cs_2CO_3 (35 mmol, 11.4 g) and morpholine **4** (3 g, 34.5 mmol) were added and heated for 5 h at 80 °C. Once complete, the reaction mixture was cooled to room temperature (monitored by TLC), washed with water (25 mL) and extracted with ethyl acetate (3×25 mL) followed by separation of organic layer. Next, a brine solution (25 mL) was used to wash the organic layer and it was dried over Na_2SO_4. Finally, the organic layer was filtered and concentrated using the rotary evaporator to obtain compound **(5)**.

*General procedure for the synthesis of N-[1-(3-Fluoro-4-morpholinophenyl)-1H-tetrazol-5-yl]-amide derivatives (**7a-l**):*

1-(3-Fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine **(5)** (246 mg, 0.93 mmol) was dissolved in CH_2Cl_2 (5 mL), cooled to 0 °C and added DIPEA (13 mg, 1 mmol) followed by 1 mmol of acid chlorides **(6a-l)**. The reaction mixture was stirred at room temperature for 10 h and extracted with ethyl acetate

(3×10 mL). The combined organic layer was washed with brine solution (10 mL), dried over Na_2SO_4 and concentrated in vacuo to obtain the corresponding tetrazole derivatives **7a-l.**

N-[1-(3-Fluoro-4-morpholinophenyl)-1H-tetrazol-5-yl]benzamide (7a): The compound **7a** was prepared according to general procedure by utilizing benzoyl chloride. Pale pink solid; Yield: 85 %; M.P: 250-252 °C; ^{1}H NMR (400 MHz, DMSO) δ: 11.05 (brs, 1H, NH), 8.09 (d, *J* = 7.2 Hz, 2H, ArH), 7.66 (d, *J* = 7.6 Hz, 1H, ArH), 7.55 (t, *J* = 7.6 Hz, 2H, ArH), 7.39-7.34 (m, 2H, ArH), 6.99 (t, *J* = 8.8 Hz, 1H, ArH), 3.89 (t, *J* = 4.8 Hz, 4H, CH_2), 3.16 (t, *J* = 4.8 Hz, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.0, 157.4, 155.4, 145.4, 144.0, 142.9, 132.3; 127.2, 132.3, 127.2, 124.8, 124.1, 123.9, 121.3, 120.4, 54.9, 50.8; IR (KBr, cm^{-1}): 3270, 3081, 2982, 2921, 2839, 1691, 1583, 1546, 1448, 1344, 1265, 1103, 936; m/z (ESI–MS): calculated for $C_{18}H_{18}N_6O_2F$ $(M+H)^+$: 369.36

N-(1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-yl)-3,3-dimethylbutanamide (7e): The compound **7e** was prepared according to general procedure by utilizing 3,3-dimethyl butyryl chloride **6e**. Pale yellow solid; Yield: 79 %; M.P: 138–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.24 (br s, 1H), 7.61-7.39 (m, 2H, ArH), 7.35 (t, *J* = 8.8, 1H, ArH), 4.24 (d, *J* = 4.8 Hz, 4H, CH_2), 3.52 (t, *J* = 4.8 Hz, 4H, CH_2), 2.87 (s, 2H, CH_2), 1.58 (s, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ:162.2, 154.8, 135.0, 134.9, 134.0, 133.2, 128.0, 127.5, 125.2, 52.2, 47.4, 46.5, 25.5, 21.5, 18.1; IR (KBr, cm^{-1}): 3433, 3076, 2925, 2852, 1741, 1658, 1607, 1526, 1474, 1351, 1207, 917; m/z (ESI–MS) 363.23 $[M + H]^+$.

N-(1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-yl)-2-nitrobenzamide (7k): The compound **7k** was prepared according to general procedure by utilizing 2-nitrobenzoyl chloride **6k.** Yield: 72 %; M.P: 243–245 °C; ^{1}H NMR (400 MHz, DMSO) δ: 11.09 (brs, 1H), 8.12 (d, *J* = 7.7 Hz, 2H, ArH), 7.66 (t, *J* = 7.8Hz, 1H, ArH), 7.61 (d, *J* = 7.8 Hz, 1H, ArH), 7.39-7.32 (m, 2H, ArH), 6.97 (t, *J* = 8.9 Hz, 1H, ArH), 3.86 (t, *J* = 4.9 Hz, 4H, CH_2), 3.16 (t, *J* = 4.9 Hz, 4H, CH2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.9, 151.8, 142.2, 139.1, 135.8, 130.0, 129.9, 129.6, 129.0, 131.7, 128.7, 124.2, 123.7, 121.2, 120.7, 59.0, 45.8; IR (KBr, cm^{-1}): 3438, 3070, 2923, 2840, 1691, 1543, 1478, 1265, 1132, 945; m/z (ESI–MS) 414.24 $[M + H]^+$.

Procedure for Antioxidant Activity:

Ai Lan Chew *et al.* method [35] was adopted to find out the free radical scavenging activity of the numerous extracts for 2,2-Diphenyl-1-picryl hydrazyl (DPPH). Crude extracts of different concentrations: 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL were placed in DMSO. 1 mL of each concentration was mixed with 4 mL of 0.004 % (w/v) DPPH solution prepared in CH_3OH. The reaction mixture was left in the dark for 30 min incubation. CH_3OH was used as control and ascorbic acid was employed as positive control. The absorbance was calculated at 517 nm. The following formula was used to find out the DPPH scavenging activity (%):

$$\text{DPPH scavenging activity (\%)} = [(A\text{o} - A\text{s}) / A\text{o}] \times 100,$$

where, Ao = absorbance of the control, As = absorbance of the prepared sample.

Methodology and Programs of Quantum Chemical Calculations:

In this work, we utilized advanced quantum chemical calculations performed using state-of-the-art Gaussian 09 computational software [36, 37]. This software is known for its exceptional accuracy in modeling molecular structures and properties. Our calculations were executed using the [B3LYP/6-31G(d, p)] level of theory, a variant of Density Functional Theory (DFT). This method is highly regarded for its ability to provide accurate insights into molecular behaviour [37, 38]. The chosen level of theory ensures that our simulations capture intricate electronic interactions and structural features, which are critical for understanding the chemistry and properties of the molecules under study. Our study benefits from the robust and well-established combination of Gaussian 09 and the selected DFT method.

The following formulas were used to calculate the Energy Gap, Hardness, Softness, and Chemical Potential:

1. Energy Gap (Eg):

The energy gap, or HOMO-LUMO gap, was calculated as:

$$\text{Eg} = \varepsilon\text{LUMO} - \varepsilon\text{HOMO},$$

where εLUMO is the energy of the Lowest Unoccupied Molecular Orbital (LUMO); εHOMO is the energy of the Highest Occupied Molecular Orbital (HOMO).

2. Hardness (η):

The chemical hardness was determined as:

$$\eta = (\varepsilon\text{LUMO} - \varepsilon\text{HOMO})/2.$$

It measures a molecule's ability to resist changes in electron density.

3. Softness (*S*):

Softness was assessed as the inverse of hardness:

$$S = 1/\eta.$$

It characterizes the ability of a molecule to polarize and interact with other molecules.

4. Chemical Potential (μ):

The chemical potential is related to the electronegativity and was defined as:

$$\mu = (\varepsilon LUMO + \varepsilon HOMO)/2.$$

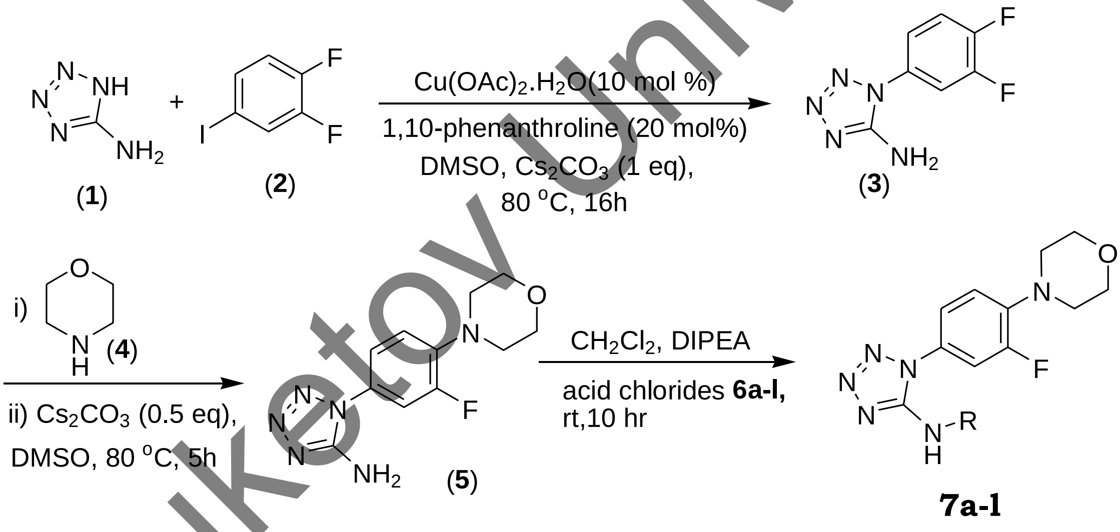
It provides insights into the reactivity and electron transfer processes within the system.

By following this methodology and using the specified quantum chemistry software, we were able to accurately calculate the energy gap, hardness, softness, and chemical potential, which are fundamental to our study. The chosen calculation method is well-supported by the scientific literature, ensuring the reliability and relevance of our results.

Results and Discussion

Chemistry:

The metal catalyzed arylation of primary amine derivatives of five- and six- membered heterocyclic compounds is of special interest, which have been challenging substrates so far represented [39, 40]. B.D. Glisic et al., [41] showed 5-aminotetrazoles electrostatic potential map, and explained that most of the electron density is located on the tetrazole ring compared to the amino group. This indicates that amino group is electron poor and therefore less nucleophilic. In this paper, we reported the synthesis of novel 1-(3-Fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine derivatives and evaluated their antioxidant properties. The complete synthetic route to produce the target tetrazole motifs **7a-l** is presented in Scheme.



Scheme. Synthesis of novel tetrazole derivatives

Initially, 5-amino tetrazole underwent regioselective *N*-arylation [42] upon reaction with 1,2-difluoro-4-iodobenzene in presence of $Cu(OAc)_2{\cdot}H_2O$ as Cu catalyst, Cs_2CO_3 as base and 1,10-phenanthroline as ligand at 85 °C for 18 h, to obtain the intermediate 1-(3,4-difluorophenyl)-1H-tetrazol-5-amine (**3**). Next the intermediate (**3**) on reaction with morpholine (**4**) in DMSO at 80°C for 5 h produced the precursor 1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine **(5).** Finally, the intermediate (**5**) by the reaction with different acid chlorides **6a-l** in CH_2Cl_2 and DIPEA at room temperature for about 10 h yielded the target tetrazole motifs **7a-l** in 72-91 % of yields. The total synthetic results are presented in Table 1.

Table 1

Synthetic results of the targeted tetrazoles 7a-j

Entry	RCOCl, R=	Reaction Time	Product	Yield, %	M.P., °C
1	C_6H_5	7h	**7a**	85	250-252
2	$(CH_3)CH\text{-}CH_2$	8h	**7b**	82	180-182
3	$(CH_3)_2CH$	7h	**7c**	84	188-190
4	Cyclopropyl	8h	**7d**	88	210-212
5	$(CH_3)_3C$	9h	**7e**	79	138-140
6	CH_3	6h	**7f**	87	270-272
7	C_2H_5	6h	**7g**	81	258-260
8	$2\text{-F,6-}OCH_3C_6H_3$	9h	**7h**	76	260-262
9	$2\text{-}OMeC_6H_4$	9h	**7i**	87	234-236
10	$2,6\text{-di}FC_6H_4$	9h	**7j**	74	210-212
11	$2\text{-}NO_2C_6H_4$	10h	**7k**	72	243-245
12	$2,6\text{-di}OMeC_6H_4$	8h	**7l**	91	235-237

The key step of the total protocol is the Cu promoted regioselective *N*-arylation using 1,10-phenanthroline as a ligand (Step 1). In our previous work [42] we used 1,10-phenanthroline as more efficient ligand for Cu catalyzed regio-selective *N*-arylation (C-N coupling). Moreover, efficient *C-N* cross coupling strategies [43-44] were reported using 1,10-phenanthroline as ligand. As it is a hydrophobic and electron-poor heteroaromatic system [45] with a central benzene ring imposing a rigid structure to hold the two nitrogen atoms in juxtaposition [46] and is capable to chelate rings with either a metal or hydrogen to form stable structures [47] it can be used a significant class of chelating ligand to catalyze various reactions. Particularly, 1,10-phenanthroline can be coordinated with Cu to form more active [CuL]. The formed [CuL] mainly catalyzed the key step for the construction of the C–X (X = C, N, O, etc.) bond to promote the whole reaction [48]. However, the tandem reaction is complex and it is difficult to recognize the precise mechanistic role of [CuL], for which the [CuL]-catalyzed transformation may occur at the initial, intermediate or final step. Next, intermediate (**3**) reacts with morpholine (**4**) using Cs_2CO_3 as a base (HF acceptor) to form the key precursor 1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine **(5).** It is noteworthy that the tetrazole ring exhibits strong electron-withdrawing inductive effect (–I effect), which is more effective than its weak mesomeric effect (+M effect), behaves as a deactivating group [49-50] and the flow of the electrons is towards the ring. This may be the reason for the selective substitution of florine at the *para* position to form the precursor (**5**). Finally, the intermediate (**5**) reacts with various acid chlorides **6a-j** to form the targeted tetrazole amides **7a-l** in good yields.

The ^1H NMR spectrum of the compound (**5**) protons resonating at δ 7.74 ppm (multiplet, 2H) and δ 7.52 ppm (triplet, 1H) corresponds to the 1,2,4-trrisubstituted phenyl ring. The proton signals at δ 5.10 ppm (brs, 2H), δ 4.21 ppm (triplet, 4H) and δ 3.47 ppm (triplet, 4H) represents to the following groups: $–NH_2$, $–O(CH_2)_2)$ and $–N(CH_2)_2)$ respectively. The IR spectra of the compound (**6**) showed characteristic absorption peaks at 3340, 3154, 2925, 1665 and peaks at 13840-1233 cm^{-1} signifying the presence of distinctive functional groups such as $–NH_2$, =C–H (Ar), –C=N, –C–N, and –C=C(Ar) respectively. On the basis of the above spectral data, the compound (**5**) has been characterized as 1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-amine.

Further, upon characterization of target compound **7e,** the ^1H NMR spectrum (Fig. 2) showed proton signals at δ 7.16–7.10 ppm (multiplet, 2H), δ 7.0 ppm (doublet, 1H), phenyl and 1, 2,4-trisubstitute phenyl ring. The proton signals δ 3.85 ppm (triplet, 4H) δ 3.16 ppm (triplet, 4H) represents the $–O(CH_2)_2)$ and $–N(CH_2)_2)$ groups respectively, and the signal at δ 2.28 ppm (s, 3H) indicates $–COCH_3$ protons. The IR spectra of the compound **7e** showed characteristic absorption peaks at 3454 cm^{-1} represents N–H stretching, at 1691 cm^{-1} indicates amide –C=O stretching and peaks at 1379-1254 cm^{-1} signifying the presence of distinctive functional groups such as $–NH_2$, –C=N, –C–N aromatic respectively. $(M+H)^+$ peak at 307.14 Da in the ESI mass spectra of the compound **7e** corresponding to molecular formula of $C_{13}H_{15}N_6O_2F$. Finally, the compound **7e** has been characterized as N-(1-(3-fluoro-4-morpholinophenyl)-1H-tetrazol-5-yl)acetamide on the basis of the above spectral data. Similarly the remaining compounds in the series are sufficiently characterized, and are in good relevance with the anticipated structures.

HOMO and LUMO Analysis:

The HOMO-LUMO energies and their Energy gap, Hardness, Softness, Chemical potential of the obtained chemical compounds **7a-l** were calculated by B3LYP/6-31g(d, p) method using Gaussian 09 package [36–38, 51] (Table 2, Fig. 2). Theoretical study showed that compounds 7d, 7e and 7j are more reactive than other obtained tetrazole derivatives. In this study compounds **7d, 7e, 7j** exhibited the HOMO-LUMO gap 4.7856eV, 4.7905eV, 4.89009eV, showed the lowest energy gap values and the lowest chemical potential values (–3.07254eV, –3.06173eV, –3.0166eV) with the highest chemical softness (0.41792eV, 0.41749eV,0.40899eV) values, and the lowest chemical hardness (2.3928eV, 2.3952eV, 2.44504eV), which may contribute to higher chemical reactivity than others (Table 2).

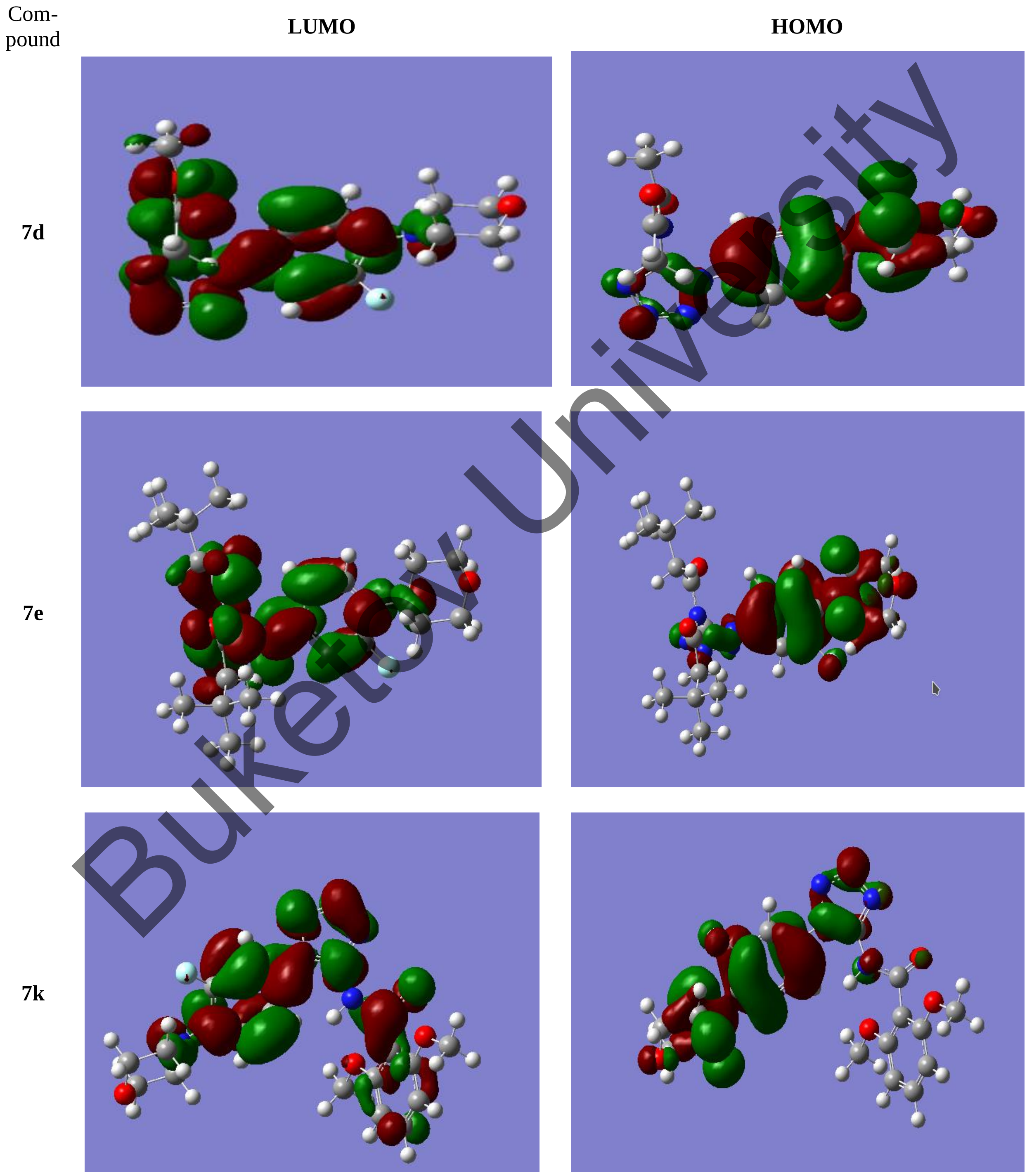


Figure 2. Frontier molecular orbital (LUMO-HOMO) of the compounds **7d, 7e** and **7k**

Table 2

HOMO, LUMO, gap, hardness, and softness of tetrazole hybrids 7d, 7e and 7k

No	Compounds	HOMO	LUMO	Gap	Hardness	Softness	Chemical potential
1	**7d**	–6.0969	–1.3113	4.7856	2.3928	0.41792	–3.07254
2	**7e**	–6.07622	–1.28572	4.7905	2.39525	0.41749	–3.06173
3	**7k**	–5.99268	–1.10259	4.89009	2.44504	0.40899	–3.0166

In general, theoretical study and HOMO-LUMO analysis of the titled compounds showed that compounds **7d**, **7e**, **7k** have the lowest energy gap and the lowest chemical potential values with the highest chemical softness and the lowest chemical hardness values, which may contribute to more high chemical activity than others.

Antioxidant Activity:

The antioxidant activity of the obtained compounds **7a-l** was assessed *in vitro* using a standard literature protocol [35]. For this intention, different extracts were examined for 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity according to the literature protocol. The crude extracts with different concentrations 25 μg/mL, 50 μg/mL, 100 μg/mL, and 200 μg/mL were tested, using ascorbic acid as a standard positive control. The results of the study are presented in Table 3.

Table 3

Antioxidant activity of titled compounds 7a-l

Sample	Antioxidant activity (%)			
	25 μg	50 μg	100 μg	200 μg
7a	23.46	25.62	29.82	34.72
7b	18.88	24.67	29.77	36.76
7c	28.87	32.84	38.54	46.45
7d	52.12	56.73	61.13	66.13
7e	54.56	58.45	66.45	72.45
7f	33.49	38.29	42.69	56.29
7g	34.72	40.42	45.98	50.22
7h	29.34	36.34	42.34	48.34
7i	34.65	37.65	39.65	42.65
7j	41.23	45.45	48.78	51.46
7k	56.56	61.45	69.45	71.89
7l	53.23	58.45	63.78	68.46
Ascorbic acid	78.74	86.06	92.81	93.25

The antioxidant activity screening results (Table 3) showed that all the obtained morpholine based tetrazole hybrids exhibited good antioxidant activity. Compounds **7a-l** exhibited a concentration-dependent increase in antioxidant activity, i.e. antioxidant activity was increased with increasing concentration. Compounds **7k** and **7b** displayed maximum and minimum antioxidant activities at the concentrations of 100 μg, 50 μg and 25μg respectively. At 200 μg concentration, the tetrazole hybrids **7e** and **7a** displayed highest and lowest antioxidant activity, respectively. Similarly, compound **7k** showed almost comparable antioxidant activity to compound 7e at the concentration of 200 μg. All the remaining compounds except **7a** and **7b**, exhibited more than 45 % level of antioxidant property at 200 μg concentration. However, all obtained compounds exhibited significantly lower antioxidant activity compared to standard ascorbic acid at all concentrations tested.

Based on the results of antioxidant activity studies, it was assumed that: (i) the electron withdrawing nitro group is responsible for the moderate antioxidant activity of compound **7k**; (ii) Presence of tertiary butyl group in **7e** is accountable for antioxidant drug efficiency; (iii) similarly, the existence of electron releasing methoxy groups at 2nd and 5th positions of phenyl ring is accountable for the good antioxidant activity of compound **7l**.

Conclusions

In summary, the present work describes the synthesis of a sequence of new tetrazole derivatives (**7a-l)** via copper-promoted regioselective *N*-arylation as key step. The obtained compounds were further evaluated for *in vitro* antioxidant activity and the results revealed that compounds **7e**, and **7k** had potent antioxidant activity. Furthermore, the HOMO-LUMO analysis of the title compounds showed that compounds **7d**, **7e**, **7k** have the lowest energy gap and the lowest chemical potential values, with the highest chemical softness, the lowest chemical hardness, which possibly contributes to the higher chemical reactivity than other compounds. All obtained tetrazole analogs were characterized by ^{1}H NMR, mass and IR spectral data. In conclusion, the obtained morpholine tetrazole hybrids represent a promising, gracious surrogate and can be considered as a prime target for medicinal chemists working in the field of antioxidants.

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