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SYNTHESIS, INHIBITORY PROPERTIES ON THE METABOLIC ENZYMES AND ANTIOXIDANT ACTIVITY OF A NEW SERIES BENZIMIDAZOLE DERIVATIVES

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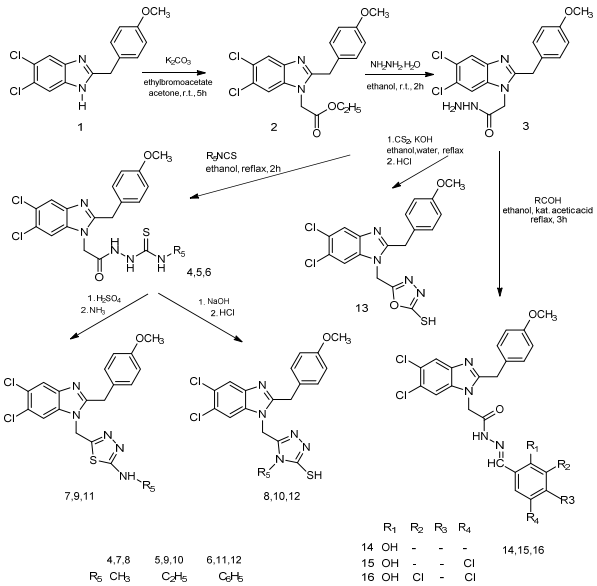
In this study, a series of new 1, 2-disubstituted benzimidazole was synthesized starting from 5, 6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazole compound which was converted to the ester and hydrazide derivative, respectively. Then, the carbothioamide derivative compounds were obtained by using several isothiocyanates. The thiadiazole derivative compounds were synthesized by closing the intramolecular ring of the carbothioamide derivatives in the presence of conc. H_2SO_4. And also intramolecular ringing of carbothioamides in basic medium resulted in the formation of the 1,2,4-triazole derivative compounds. Moreover, 5-((5,6-dichloro-2-(4-methoxybenzyl)-1H-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol compound was syntesized in basic media via reaction of hydrazide derivative with CS_2. The treatment of hydrazide derivative with various salicylic aldehydes gave Schiff-based benzimidazole derivative compounds. The structural characterization of all synthesized compounds was elucidated by elemental analyses, ^{1}H NMR, ^{13}C NMR and mass spectral methods. They were also screened for their antioxidant activity and inhibitory properties on the metabolic enzymes such as urease and xanthine oxidase.



INTRODUCTION

The structural similarity of the benzimidazole core to the purine base makes it easy to interact with living systems. The presence of this valuable structure in the synthesis and construction of the molecules of new drug attracts attention due to a wide range of pharmacological activities such as antimicrobials, antivirals, anticancer, anti-inflammatory, antioxidants, anticoagulants, antidiabetic and psychoactive agents.[1–9] Similarly, synthesis of constructure of the 1,2,4-triazole, oxadiazole, 1,3,4-thiadiazole rings, carbotioamide and Schiff based molecules attracts considerable attention of medicinal chemistry owing to their diverse applications as anticancer, antibacterial, antidepresant, antioxidant.[10–13] Also, several literature surveys show that these structures at the 1- and 2- position of the benzimidazole core markedly affect pharmacological activity.[14–16]

The results of the studies in the literature have encouraged us to modify the benzimidazole

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compound with different substituents. Thus, we have combined the 5,6-dichloro-2-(4-methoxybenzyl)-1*H*-benzimidazole compound with the 1,2,4-triazole, 1,3,4-thiadiazol, 1,3,4-oxadiazole rings, carbothioamide and Schiff base structure in order to observe the effects on antioxidant, xanthine oxidase and urease inhibition results.

RESULTS AND DISCUSSION

1. Chemistry

Our previous studies show that iminoester compounds for the synthesis of benzimidazole deriative compounds could be useful starting compounds.[11] In this study, the starting compound ethyl 2-(4-methoxyphenyl)acetimidate hydrochloride was prepared according to the known literature procedure.[11] To synthesize 5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazole compound (**1**), iminoester hydrochloride was reacted with 4,5-dichlorobenzene-1,2-diamine in high yield in absolute methanol. Then, treatment of compound **1** with ethyl bromacetate in absolute acetone gave acetate derivative compound, ethyl [5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetate (**2**). And, this ester compound was converted to hydrazine compound, 2-[5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetohydrazide (**3**) by the reaction of compound **2** with hydrazine hydrate. In the next steps of the synthesis, the carbothioamide derivative compounds (**4, 5, 6**) were obtained by the nucleophilic addition of compound **3** to methylisothiocyanate (for **4**), ethylisothiocyanate (for **5**) or phenylisothiosyanate (for **6**). Then, 1,2,4-triazole compounds (**8**, **10**, **12**) are formed by the intramolecular ring closure of carbothioamide compounds in the presence of sodium hydroxide. Moreover, the cyclisation of the same intermediates **(4-6)** in the presence conc. and cold. sulfuric acid produced 1,3,4-thiadiazol compounds (**7**, **9**, **11**). The treatment of hydrazide derivative compound (**3**) with CS_2 in the presence of KOH resulted in the formation of 5-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (**13**). In the final step, the synthesis of three different Schiff base derivatives (**14-16**) was obtained by the reaction of compound **3** with several salicyl aldehydes in ethanol in the presence of AcOH. In ^{1}H-NMR spectra of these compounds (**14-16**), due to cis- and trans-conformers of the amide structure, the some proton signals of these compounds were observed in duplicate sets.[17-19] The synthesis routes for the target compounds are shown in scheme 1. The ^{1}H-NMR, ^{13}C-NMR and elemental analysis spectral results of the synthesized compounds were obtained. They were consistent with the constructions of all the compounds.

2. CUPRAC Antioxidant Activity Assay

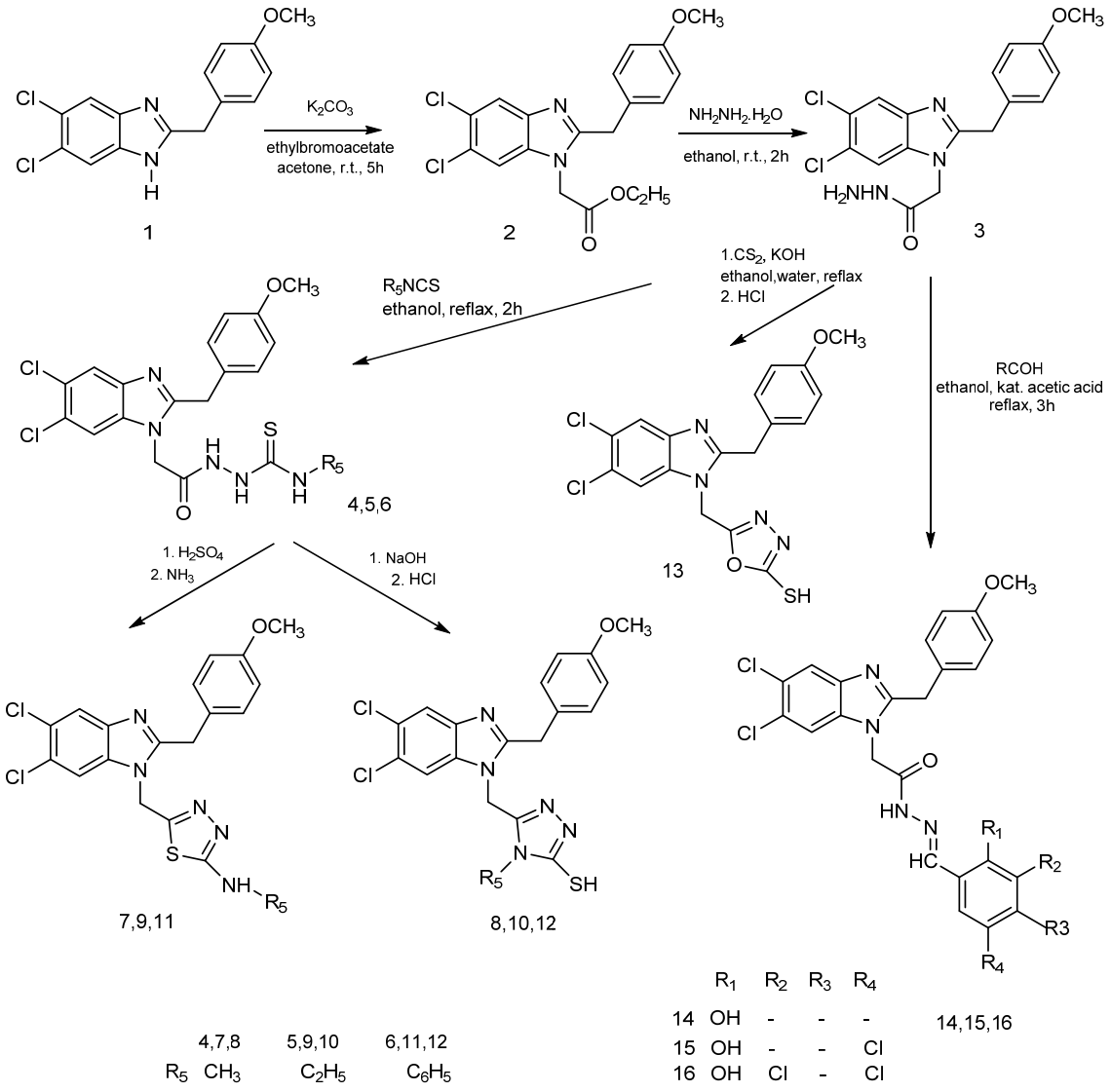
According to the CUPRAC method, antioxidant activity of the benzimidazole derived compounds can be listed as follows, from the most active molecules to the least active molecules, **10**>**5**>**3**>**12**>**13**>**6**>**8**>**4**>**16**>**15**>**2**>**7**>**11**>**1**>**14**>**9** (Figure 1). Similarly to our TEAC results, researchers reported that triheterocyclic compounds containing 1,3,4-thiadiazole and 1,2,4-triazole groups and benzimidazole derivatives containing a triazole nucleus were highly active in Cuprac assay.[15,16]

3. DPPH$^{\bullet}$ Scavenging Assay

The total radical scavenging capacity of the benzimidazole derived compounds was determined. Because of having the lowest SC_{50} value, compound **10** was the best among the all synthesized compounds and had the highest TEAC value. Also, compound **3** and **5** showed good DPPH radical scavenging activity. Also, compounds **3**, **5**, **10** and **12** showed more scavenging activity than BHT. Besides these efficient results, some of them, which were the compounds **4**, **6**, **8**, **12** and **13** showed moderate radical scavenging activity (Figure 2). The compounds **1**, **2**, **9**, **11** and **14** that had weak antioxidant activity to Cuprac method exhibited weak radical scavenging activity to DPPH method (Figure 2). Researchers reported that benzimidazole derivatives containing a triazole nucleus, oxadiazole and thiosemicarbazide were highly active in DPPH method with very effective SC50 values.[16,18]

4. ABTS$^{\bullet+}$ Scavenging Assay

In this ABTS radical scavenging assay, the compounds **3, 4, 5, 6, 8, 10, 12** and **13** showed fairly well scavenging activity at the 6.0 µg/mL final concentration (Table 1)**.** In addition, at 1.5 µg/mL final concentration, it was found that the compounds **3**, **8**, **10** and **13** scavenged more than half of the ABTS$^{\bullet+}$ radical. Benzimidazole derivative compounds containing some different groups, such as 1,3,4-thiadiazole, 1,2,4-triazole,[15] 1,3,4-oxadiazole rings, salicyl, thiosemicarbazide structure[18] and fluoro[20] have been reported to good ABTS•+ radical scavenging activity.



Scheme 1 – The synthesis pathway for the preparation of the target compounds.

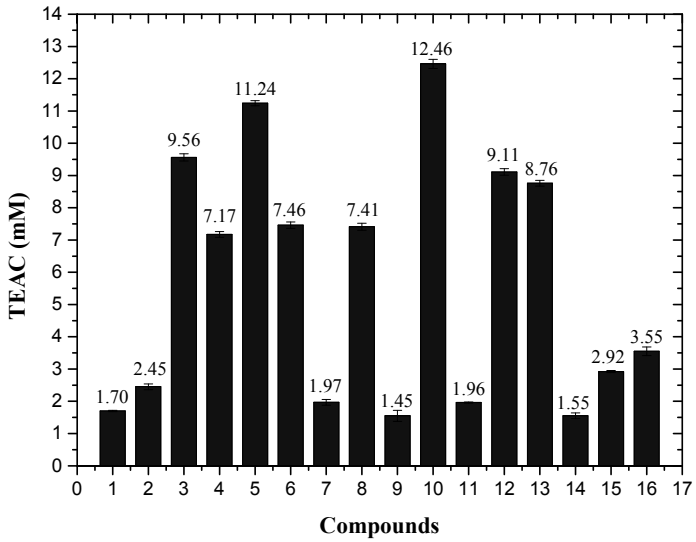


Fig. 1 – Antioxidant activity values to CUPRAC method of synthesized compounds.

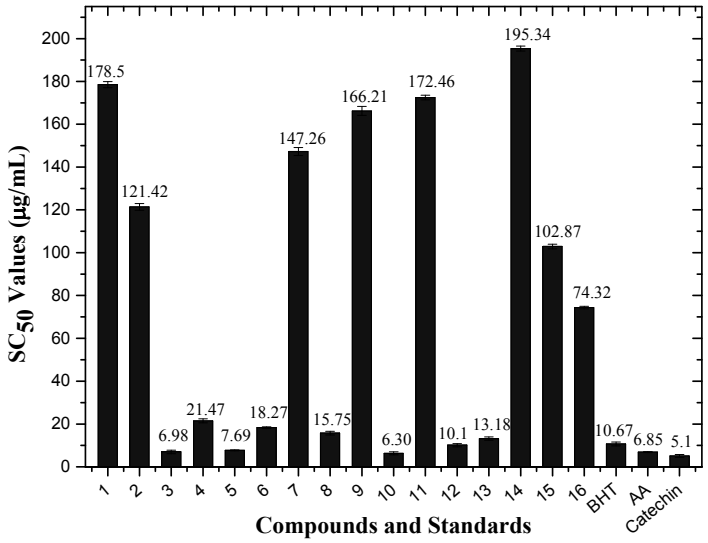


Fig. 2 – SC_{50} values to DPPH method of synthesized compounds and standards. AA, Ascorbic acid.

Table 1

$ABTS^{\bullet+}$ radical scavenging activity values of the synthesized compounds

Compounds and Standards	ABTS method, % scavenging				
	6.0 (µg/mL)	3.0 (µg/mL)	1.5 (µg/mL)	0.75 (µg/mL)	0.375 (µg/mL)
1	15.21%	12.39%	10.35%	9.90%	7.43%
2	19.43%	14.32%	11.49%	9.66%	6.33%
3	90.31%	88.00%	52.77%	30.46%	20.00%
4	88.92%	68.31%	40.00%	23.23%	16.31%
5	90.62%	76.00%	47.38%	27.54%	13.08%
6	88.77%	64.92%	44.15%	26.31%	20.31%
7	16.99%	14.57%	12.58%	10.08%	7.54%
8	90.46%	87.38%	55.38%	31.54%	20.31%
9	14.89%	12.06%	10.31%	8.99%	7.76%
10	90.77%	75.69%	53.69%	33.38%	22.46%
11	14.89%	12.59%	10.87%	9.85%	6.89%
12	82.00%	61.85%	47.08%	27.69%	17.54%
13	90.15%	87.08%	69.69%	40.77%	25.69%
14	15.69%	13.17%	10.86%	8.67%	6.09%
15	16.44%	13.58%	10.51%	8.39%	6.6%
16	23.45%	19.34%	15.88%	11.79%	6.19%
Catechin	90.43%	86.86%	81.43%	42.86%	20.00%
BHT	88.19%	42.92%	12.11%	5.21%	2.40%
Ascorbic Acid	90.14%	51.13%	35.89%	15.10%	6.48%

Table 2

Results of % residual XO activity and IC_{50} values of the synthesized chemical compounds

Residual XO activity %		
Compound	(60 µg/mL)	IC_{50} (µg/mL)
Control	100.00	-
11	8.94±1.19	16.75±0.68
Allopurinol (8.0 µg/mL)	1.13 ±0.15	0.62±0.03

Control, bovine milk xanthine oxidase without inhibitor; Allopurinol, positive control.

5. Anti-xanthine oxidase activity

Inhibition studies with regard for bovine milk xanthine oxidase activity had shown that compound **11** had promising activity at 60.0 µg/mL concentration, with an IC_{50} value of 16.75± 0.68 µg/mL (Table 2). In the literature, another published study, compound 6d had promising anti-XO activity with an IC_{50} value of 33.87±0.46 µM and compounds 5c, 5d and 6c showed moderate

inhibition activity.[20] Also, fluorine containing 1,2,4-triazole-5-on derivatives and benzimidazole derivatives has been reported that exhibited good inhibition activity on XO.[21,22]

6. Anti-urease activity

Initially, all synthesized compounds were examined 100 µg/mL at final concentration. Among the synthesized compounds, **4** exhibited the best inhibitory effect against urease with IC_{50} value 22.24±0.53 µg/mL. IC_{50} value of aceto-hydroxamic acid was determined as 22.00± 0.31 µg/mL. Compound **4** can be considered as potential urease inhibitors to treatment with related to *Helicobacter pylori* infections (Figure 3). Baltas vd., reported IC_{50} values of thiourea, compounds 5b and 5c as 11.91±0.33, 7.41±0.13 and 10.48±0.15 µg/mL, respectively.[21] Researchers emphasized that 9b exhibited the best inhibitory effect against urease with IC_{50} value 28.89± 0.11 µM. Compound 9b inhibited urease activity by 36.07±0.41%, 68.29±0.09% and 98.43±0.28 at concentrations of 10.94, 21.87 and 43.75 µg/mL, respectively.[22]

EXPERIMENTAL

1. Chemistry

All reagents were purchased from Merck, Sigma-Aldrich and Fluka. The melting points of the synthesized compounds were determined on a Stuart SMP model melting point determination device and were uncorrected. The progress of the reactions was monitored by thin layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. ^{1}H-NMR and ^{13}C-NMR spectra were performed on Varian-Mercury 400 MHz (^{1}H, 400 MHz; ^{13}C 100 MHz) spectrometer using TMS as internal reference and DMSO-d_6 as solvent. All chemical shifts are reported in ppm. The Mass spectra were obtained on Thermo Scientific Quantum Access max LC-MS spectrometer.

1.1. Procedure for the preparation of 5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazole **(1)**

The precursor material, 5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazole, **1**, was prepared according to literature procedure.[16] Final solid product was filtered off and purified three times by crystallization from ethanol-water (1:1) to afford compound **1**. Yield: 85 %; m.p: 198-199 °C; ^{1}HNMR (DMSO) δ: 3.69 (3H, s, OCH_3), 4.09 (2H, s, CH_2), 6.86 (2H, d, ArH, *J* = 8 Hz), 7.22 (2H, d, ArH, *J* = 8 Hz), 7.71 (2H, s, ArH), 12.52 (1H, s, NH); ^{13}CNMR (DMSO) δ: 34.40 (CH_2), 55.50 (OCH_3), ArC [114.40, 124.13, 129.90, 130.32, 157.42, 158.52] ppm; ESI-MS: m/z 307.10 (M+, 100%).

1.2. Procedure for the preparation of ethyl [5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetate **(2)**

The compound **1** (0.010 mol) in absolute acetone (15 mL) was stirred with K_2CO_3 (0.030 mol) at room temperature for 1 hour. Then, the mixture was added with ethylbromoacetate (0.011 mol) and stirred for a further 4 hours. The mixture was added with plenty of water and the resulting white solid was filtered and purified from ethanol-water (1:1) to afford compound **2**. Yield: 81 %; m.p: 177-178 °C; ^{1}HNMR (DMSO) δ: 1.10 (3H, t, CH_3, *J* = 8 Hz), 3.69 (3H, s, OCH_3), 3.95 (2H, q, CH_2, *J* = 8 Hz), 4.16 (2H, s, CH_2), 5.16 (2H, s, CH_2), 6.83 (2H, d, ArH, *J* = 8 Hz), 7.16 (2H, d, ArH, *J* = 8 Hz), 7.87 (1H, s, ArH), 7.91 (1H, s, ArH); ^{13}CNMR (DMSO) δ: 14.30 (CH_3), 32.45 (CH_2), 45.19 (CH_2), 55.48 (OCH_3), 61.68 (OCH_2), ArC [112.61, 114.30, 120.20, 124.59, 124.91, 127.97, 130.38, 135.86, 142.09, 157.32, 158.50, 167.78] ppm; ESI-MS: m/z 393.29 (M+, 100%).

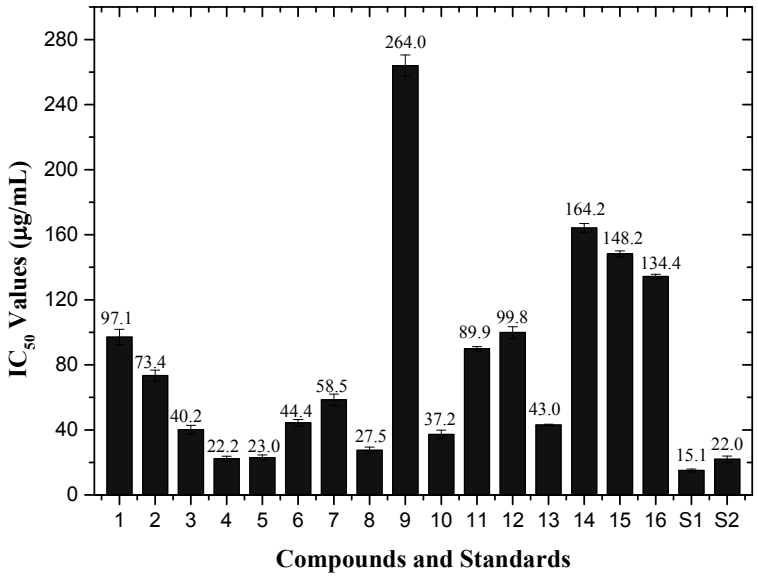


Fig. 3 – IC_{50} values of the synthesized compounds against *Jack bean* urease.
Control, *Jack bean* urease without inhibitor; Thiourea (S1) and acetohydroxamic acid (S2) were used as standard inhibitors.

1.3. Procedure for the preparation of 2-[5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetohydrazide **(3)**

The ester compound **(3)** (0.010 mol) in absolute ethanol (15 mL) was stirred at room temperature for 5 hours with portionwise addition of hydrazine hydrate (0.040 mol) (4× 0.5 mL).The solid formed after the reaction was filtered and dried. The resulting solid was purified by washing from boiling ethanol and acetone respectively to afford compound **3**. Yield: 65 %; m.p: 213-214°C; ^{1}HNMR (DMSO) δ: 3.70 (3H, s, OCH_3), 4.06 and 4.14 (2H, s, CH_2), 4.31 and 4.54 (1H, s, NH), 4.82 and 5.17 (2H, s, CH_2), 6.85 (2H, d, ArH, *J* = 8 Hz), 7.18 (2H, d, ArH, *J* = 8 Hz), 7.80 (2H, d, ArH, *J* = 4 Hz), 9.46 (1H, s, NH); ^{13}CNMR (DMSO) δ: 32.50 (CH_2), 45.12 (NCH_2), 55.48 (OCH_3), ArC [112.42, 114.32, 120.13, 124.41, 124.69, 128.34, 130.45, 135.80, 142.16, 157.65, 158.49, 165.96] ppm; ESI-MS: m/z 380.31 (M+H, 100%).

1.4. General method for the synthesis of the carbothioamide derivative compounds **(4-6)**

A mixture of compound **3** (0.010 mol) and methylisothiocyanate (for **4**), ethylisothiocyanate (for **5**) or phenylisothiosyanate (for **6**) was refluxed in absolute ethanol for 3-4 h (controlled with TLC). The mixture was cooled at room temparature. Then, this crude product was filtered off and recrystallized from ethanol-water (2:1) to afford the desired product.

2-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}-N-methylhydrazinecarbothioamide **(4).** Yield: 85 %; m.p: 225-226 °C; ^{1}HNMR (DMSO) δ: 2.89 (3H, s, CH_3), 3.70 (3H, s, OCH_3), 4.14 (2H, s, CH_2), 4.93 (2H, s, NCH_2), 6.86 (2H, d, ArH), 7.17 (2H, s, ArH), 7.71-7.82 (2H, m, ArH), 8.06, 9.30 and 10.22 (3H, s, NH); ^{13}CNMR (DMSO) δ: 31.38 (NCH_3), 32.44 (CH_2), 45.13 (NCH_2), 55.50 (OCH_3), ArC [112.55, 114.38, 120.15, 124.51, 124.75, 128.33, 130.35, 130.44, 135.74, 142.16, 157.70, 158.52], 166.58 (CO), 172.84 (CS) ppm; ESI-MS: m/z 453.36 (M+H, 100%).

2-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}-N-ethylhydrazine carbothioamide **(5)**.
Yield: 88 %, m.p: 231-232 °C; ^{1}HNMR (DMSO) δ: 1.06 (3H, t, CH_3, *J*= 8 Hz), 3.46 (2H, q, CH_2, *J*= 8 Hz), 3.70 (3H, s, OCH_3), 4.14 (2H, s, CH_2), 4.94 (2H, s, NCH_2), 6.86 (2H, d, ArH, *J*= 8 Hz), 7.17 (2H, d, ArH, *J*= 8 Hz), 7.70-7.83 (2H, m, ArH), 8.06, 9.22 and 10.21 (3H, s, NH); ^{13}CNMR (DMSO) δ: 14.87 (CH_3), 32.43 (CH_2), 38.97 (NCH_2), 45.12 (NCH_2), 55.50 (OCH_3), ArC [112.57, 114.37, 120.14, 124.49, 124.74, 128.36, 130.35, 130.45, 135.76, 142.18, 157.72, 158.50], 166.46 (CO), 172.66 (CS) ppm; ESI-MS: m/z 467.23 (M+H, 100%).

2-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}-N-phenylhydrazine carbothioamide (**6**). Yield: 78 %; m.p: 196 °C; ^{1}HNMR (DMSO) δ: 3.70 (3H, s, OCH_3), 4.18 (2H, s, CH_2), 5.01 (2H, s, NCH_2), 6.80-6.87 (2H, m, ArH), 7.19 (3H, d, ArH, *J*= 8 Hz), 7.34-7.54 (4H, m, ArH), 7.83-7.87 (2H, m, ArH), 9.76 and 10.48 (3H, s, NH); ^{13}CNMR (DMSO) δ: 32.45 (CH_2), 45.19 (NCH_2), 55.50 (OCH_3), ArC[112.60, 114.37, 120.16, 124.52, 124.78, 125.81, 128.36, 130.46, 135.77, 139.32, 142.19, 157.75, 158.51], 166.53 (CO), 172.45 (CS) ppm; ESI-MS: m/z 515.51 (M+H, 78 %).

1.5. General method for the synthesis of the 1,3,4-thiadiazol derivative compounds **(7, 9 and 11)**

To the corresponding carbothioamide compounds **(4-6)** (0.010 mol), cold and concentrated sulfuric acid (0.064 mol) was added dropwise at 0-5ºC for 20 min. Then, the mixture was stirred at room temperature for an additional 45 min., the resulting mixture was poured into cold water and alkalized to pH 7-8 with ammonia. The precipitated product was filtered, washed with plenty of water and recrystallized from ethanol to afford the desired compound.

5-((5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl)methyl)-N-methyl-1,3,4-thiadiazol-2-amine **(7).** Yield: 54 %; m.p: 285-286 °C; ^{1}HNMR (DMSO) δ: 2.87 (3H, s, NCH_3), 3.66 (3H, s, OCH_3), 4.38 (2H, s, CH_2), 5.87 (2H, s, NCH_2), 6.87 (2H, d, ArH, *J*=8 Hz), 7.24 (2H, d, ArH, *J*=8 Hz), 7.60-8.12 (2H, m, ArH), 10.37 (1H, s, NH); ^{13}CNMR (DMSO) δ: 31.95 (CH_3), 32.22 (CH_2), 42.80 (CH_2), 55.90 (OCH_3), ArC [112.68, 113.56, 119.25, 125.32, 126.53, 129.70, 134.14, 135.86, 138.14, 152.13, 155.96, 156.90, 170.43] ppm; ESI-MS: m/z 434.32 (M+, 100%).

5-((5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl)methyl)-N-ethyl-1,3,4-thiadiazol-2-amine (**9**)**.** Yield: 61 %; m.p: 270-271 °C; ^{1}HNMR (DMSO) δ: 1.20 (3H, t, CH_3), 3.15 (2H, q, CH_2), 3.65 (3H, s, OCH_3), 4.24 (2H, s, CH_2), 5.80 (2H, s, NCH_2), 6.98 (2H, d, ArH, *J*=8 Hz), 7.12 (2H, d, ArH, *J*=8 Hz), 7.56-7.89 (2H, m, ArH), 10.22 (1H, s, NH); ^{13}CNMR (DMSO) δ: 15.62 (CH_3), 32.10 (CH_2), 39.87 (CH_2), 42.72 (CH_2), 55.39 (OCH_3), ArC [112.66, 113.54, 119.25, 125.32, 126.53, 129.69, 134.13, 135.84, 138.14, 152.10, 155.98, 156.90, 169.36] ppm; ESI-MS: m/z 449.25 (M+H, 54 %).

5-((5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine **(11).**
Yield: 56 %; m.p: 279-280 °C; ^{1}HNMR (DMSO) δ: 3.67 (3H, s, OCH_3), 4.42 (2H, s, CH_2), 5.94 (2H, s, NCH_2), 6.88 (2H, d, ArH, *J*=8 Hz), 6.90 (1H, s, ArH), 7.12 (1H, s, ArH), 7.25 (1H, s, ArH), 7.45-7.55 (3H, m, ArH), 7.70-8.17 (3H, m, ArH), 10.52 (1H, s, NH); ^{13}CNMR (DMSO) δ: 32.35 (CH_2), 42.67 (NCH_2), 55.95 (OCH_3), ArC [112.62, 114.02, 116.78, 119.02, 120.54, 124.78, 127.02, 128.53, 129.72, 133.23, 35.54, 140.80, 142.18, 156.01, 156.94, 165.78] ppm; ESI-MS: m/z 496.38 (M+, 100%).

1.6. General method for the synthesis of the 1,2,4-triazole derivative compounds **(8, 10 and 12)**

A solution of the carbothioamide derivatives **(4-6)** (0.010 mol) in ethanol (15 mL) was allowed to reflux in the presence of 2N 15 mL NaOH for 4h. Then, the mixture was cooled at room temperature and acidified to pH 6 with 37 % HCl. The precipitated product was filtered, washed with plenty of water and recrystallized from ethanol to afford the desired compound.

5-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-methyl-4H-1,2,4-triazole-3-thiol **(8).** Yield: 80%; m.p: 260-261 °C; ^{1}HNMR (DMSO) δ: 3.33 (3H, s, CH_3), 3.44 (3H, s, OCH_3), 4.18 (2H, s, CH_2), 5.60 (2H, s, NCH_2), 6.77 (2H, d, ArH, *J*=8 Hz), 7.14 (2H, d, ArH, *J*=8 Hz), 7.85 (1H, s, ArH), 7.94 (1H, s, ArH), 13.40 (1H, s, SH); ^{13}CNMR (DMSO) δ: 30.31(CH_3), 32.25 (CH_2), 39.33-40.58 (DMSO+CH_2), 55.47 (CH_3), ArC [112.69, 114.14, 120.26, 124.69, 124.97, 126.11, 130.31, 135.72, 142.21, 148.40, 157.58, 158.46, 167.84] ppm; ESI-MS: m/z 434.45 (M+, 34%).

5-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-ethyl-4H-1,2,4-triazole-3-thiol **(10).** Yield: 68 %; m.p: 271-272 °C; ^{1}HNMR (DMSO) δ: 1.17 (3H, t, CH_3,

J= 8 Hz), 3.68 (3H, s, OCH_3), 3.95 (2H, q, CH_2, *J*= 8 Hz), 4.21 (2H, s, CH_2), 5.64 (2H, s, NCH_2), 6.75 (2H, d, ArH, *J*= 8 Hz), 7.09 (2H, d, ArH, *J*= 8 Hz), 7.87 (1H, s, ArH), 7.94 (1H, s, ArH), 13.41 (1H, s, SH); ^{13}CNMR (DMSO) δ: 13.49 (CH_3), 32.45 (CH_2), 38.95 (CH_2), 39.27 (CH_2), 55.47 (CH_3), ArC [112.70, 114.13, 120.30, 124.73, 125.04, 127.80, 130.24, 135.81, 142.13, 147.66, 157.39, 158.47, 167.29] ppm; ESI-MS: m/z 448.56 (M+, 100%).

5-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-phenyl-4H-1,2,4-triazole-3-thiol **(12).**
Yield: 76 %; m.p: 277-278 °C; ^{1}HNMR (DMSO) δ: 3.69 (3H, s, OCH_3), 4.01 (2H, s, CH_2), 5.27 (2H, s, NCH_2), 6.78 (2H, d, ArH, *J*=8 Hz), 7.00 (2H, d, ArH, *J*=8 Hz), 7.39-7.52 (5H, m, ArH), 7.71 (1H, s, ArH), 7.80 (1H, s, ArH), 13.74 (1H, s, SH); ^{13}CNMR (DMSO) δ: 32.38 (CH_2), 39.29-40.54 (DMSO +CH_2), 55.52 (CH_3), ArC [112.68, 114.26, 120.09, 124.62, 124.93, 127.69, 128.46, 129.88, 130.16, 133.26, 135.62, 141.87, 147.50, 156.98, 158.48, 168.91] ppm; ESI-MS: m/z 497.34 (M+H, 67%).

1.7. Procedure for the preparation of 5-{[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol **(13)**

Compound **3** (0.010 mol) and CS_2 (0.011 mol) were refluxed in KOH solution in ethanol-water (20 mL: 20 mL) for 5 h. Then, the mixture was acidified with %37 HCl. The resulting solid particles were filtered and washed with plenty of water. The product was purified by crystallization from ethanol to afford compound **13**. Yield: 80%; m.p: 248-249 °C; ^{1}HNMR (DMSO) δ: 3.69 (3H, s, OCH_3), 4.25 (2H, s, CH_2), 5.68 (2H, s, NCH_2), 6.78 (2H, d, ArH, *J* = 8 Hz), 7.12 (2H, d, ArH, *J* = 8 Hz), 7.88 (1H, s, ArH), 7.99 (1H, s, ArH), 14.33 (1H, brs, SH); ^{13}CNMR (100 MHz, DMSO) δ: 32.32 (CH_2), 39.01 (NCH_2), 55.46 (OCH_3), ArC [112.67, 114.23, 120.41, 125.05, 125.30, 127.72, 130.29, 135.47, 142.07, 157.26, 158.51, 159.06], 178.17 (CS) ppm; ESI-MS: m/z 421.01 (M+, 100%).

1.8. General method for the synthesis of compounds **(14-16)**

Compound **3** (0.010 mol) and corresponding salicyaldehydes (0.010 mol) were refluxed with acetic acid (2-3 drops) in absolute ethanol for 2-3h (controlled with TLC). Then, the mixture was cooled to room temperature and precipitated with water. The resulting solid was filtered and purified from ethanol to afford the desired product.

2-[5,6-Dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]-N'-[(2-hydroxyphenyl)methylene] acetohydrazide **(14).**
Yield: 72 %; m.p: 274-275°C; ^{1}HNMR (DMSO) δ: 3.63 and 3.68 (3H, s, OCH_3, trans-cis conformer (% 36 / % 64)), 4.14 and 4.18 (2H, s, CH_2, cis-trans conformer (% 68 / % 32)), 5.0 and 5.44 (2H, s, NCH_2, trans-cis conformer (% 36 / % 64)), 6.79-6.91 (4H, m, ArH), 7.13-7.25 (3H, m, ArH), 7.53-7.92 (3H, m, ArH), 8.32 and 8.41 (1H, s, C=H, cis-trans conformer (% 55 / % 45)), 10.04 and 10.85 (1H, s, OH, cis-trans conformer (% 65 / % 35)), 11.64 and 11.89 (1H, s, NH, cis-trans conformer (% 62 / % 38)); ^{13}CNMR (DMSO) δ: 32.67 (CH_2), 45.15 (NCH_2), 55.43 (OCH_3), ArC [112.79, 114.27, 116.58, 119.77, 119.99, 120.54, 124.26, 124.65, 126.94, 128.27, 130.42, 131.77, 136.39, 141.97, 142.18, 156.87, 157.73, 158.46], 167.82 (CO) ppm; ESI-MS: m/z 484.28 (M+H, 100%).

N'-[(5-chloro-2-hydroxyphenyl)methylene]-2-[5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl] acetohydrazide ***(15).***
Yield: 80 %; m.p: 278-279 °C; ^{1}HNMR (DMSO) δ: 3.63 and 3.68 (3H, s, OCH_3, trans-cis conformer (% 27 / % 73)), 4.14 and 4.17 (2H, s, CH_2, cis-trans conformer (% 68 / % 32)), 5.03 and 5.49 (2H, s, NCH_2, trans-cis conformer (% 25 / % 75)), 6.82-6.93 (3H, m, ArH), 7.15-7.28 (3H, m, ArH), 7.61- 7.90 (3H, m, ArH), 8.27 and 8.37 (1H, s, C=H, cis-trans conformer (% 74 / % 26)), 10.36 and 10.94 (1H, s, OH, cis-trans conformer (% 75 / % 25)), 11.72 and 11.96 (1H, s, NH, cis-trans conformer (% 76 / % 24)); ^{13}CNMR (DMSO) δ: 32.62 (CH_2), 45.24 (NCH_2), 55.42 (OCH_3), ArC [112.76, 114.25, 118.38, 119.98, 122.47, 123.71, 124.28, 124.65, 128.26, 130.45, 131.15, 136.35, 139.92, 142.14, 155.63, 157.79, 158.47], 168.10 (CO) ppm; ESI-MS: m/z 517.82 (M+, 78%).

N'-[(3,5-dichloro-2-hydroxyphenyl)methylene]-2-[5,6-dichloro-2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetohydrazide **(16).**
Yield: 78 %; m.p: 280-281°C; ^{1}HNMR (DMSO) δ: 3.61 and 3.68 (3H, s, OCH_3, trans-cis conformer (% 40 / % 60)), 4.14 and 4.18 (2H, s, CH_2, cis-trans conformer (% 58 / % 42)), 5.07 and 5.52 (2H, s, NCH_2, cis-trans conformer (% 43 / % 57)), 6.81 (2H, d, ArH, *J*= 8 Hz), 7.16 (2H, d, ArH, *J*= 8 Hz), 7.61-7.91 (4H, m, ArH), 8.27 and 8.33 (1H, s, C=H, cis-trans conformer (% 58 / % 42)), 10.41 and 11.88 (1H, s, OH, trans-cis conformer (% 36 / % 64)), 11.97 and 12.23 (1H, s, NH, cis-trans conformer (% 56 / % 44)); ^{13}CNMR (DMSO) δ: 32.53 (CH_2), 45.24 (NCH_2), 55.43 (OCH_3), ArC [112.74, 114.23, 120.01, 121.96, 123.02,124.28, 124.34, 124.64, 125.82, 128.32, 130.48, 136.31, 140.80, 142.20, 157.53, 157.87, 158.45], 168.11 (CO) ppm; ESI-MS: m/z 552.33 (M+, 100%).

2. Antioxidant Activity and Radical Scavenging Assays

Antioxidant activities of the synthesized compounds were clarified using various in vitro antioxidant assays including Cupric Reducing Antioxidant Capacity (CUPRAC), ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonicacid)/Persulfate and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Catechin, Trolox® and Ascorbic acid were used as positive antioxidant.

2.1. Cupric reducing antioxidant capacity (CUPRAC) assay

In order to determine the cupric ions (Cu^{2+}), reducing ability of the synthesized compounds was determined according to the literature.[14–16] The standard curve was linear between 32 mM and 1.25 mM trolox (r2=0.9989). CUPRAC values were expressed as mM Trolox® equivalent of 1 mg synthesized compound.

2.2. DPPH-Free radical scavenging assay

The DPPH radical scavenging activity of the synthesized compounds was measured using the method of Brand-Williams.[15,16,18,24,25] Briefly, 1200 microliter of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol was added to 300 µL of the synthesized compound solution in DMSO. After, in the dark for 50 min, decrease in absorbance at 517 nm was measured, using a UV-Visible spectrophotometer (1601UV-Shimadzu, Australia). The results were expressed as SC_{50} (µg/mL), which was calculated from the curves by plotting absorbance values, the SC_{50} values representing the concentration of the compound (µg/mL) required to inhibit 50% of the radicals. All determinations were carried out three times and the percentage scavenging was calculated from the formula

% Scavenging = [($OD_{control}$-OD_{test}) / ($OD_{control}$)x100].

$ABTS^{\bullet+}$ Radical Cation Decolorization Assay

The ability of the synthesized compounds to scavenge $ABTS^{\bullet+}$ radical was determined according to the literature.[15,16] ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was dissolved in water to a 7 mM concentration and diluted to get an absorbance of 0.700 ± 0.020 at 734 nm before usage. After 5 min in the dark at room temperature, the decrease of absorbance of reaction mixture containing 200 µL of compound solution and 1800 µL of the $ABTS^{\bullet+}$ solution was measured. The percentage scavenging was calculated from the formula

% Scavenging = [($OD_{control}$-OD_{test}) / ($OD_{control}$)x100].

3. Urease inhibition assay

Urease is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity. [20,21,25] The percentage remaining activity was calculated from the formula % Remaining Activity = [(ODtest)/(ODcontrol) x 100]. Thiourea (S1) and acetohydroxamic acid (S2) were used as standard urease inhibitors. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standards were assayed at the same reaction conditions.

4. *In vitro* anti-xanthine oxidase assay

The inhibition of xanthine oxidase was measured by UV spectroscopy technique at 295 nm which attributes to released uric acid from xanthine. The inhibitory activity of each compound was determined using a slight modification of the reference methods.[24–26] A well known XO inhibitor (XOI), allopurinol (Sigma–Aldrich, St. Louis, USA), was used as a positive control for the inhibition test. Residual activities were calculated by comparing to control without inhibitor. The assay was done in triplicate for calculating standard deviation. The IC_{50} value was determined as the concentration of compound that gives 50 % inhibition of maximal activity.

CONCLUSIONS

This work involves the synthesis of some novel heterocyclic benzimidazole compounds containing triazole, thiadiazole, oxadiazole rings, carbotihioamide moiety and salicyl derivatives and the results of antioxidant, anti-xanthine oxidase and anti-urease activity of these compounds. Compounds **3**, **5**, **10**, **12** (with SC_{50} values 6.98, 7.69, 6.30 and 10.10 µg/mL respectively) showed more radical scavenning activity than BHT as standard (SC_{50} value 10.67 µg/mL). Also, the compounds **4**, **6**, **8**, **12** ve **13** showed moderate activity DPPH radical scavenning activity. It is concluded that the 1,2,4-triazole ring at 2-position of benzimidazole increases the antioxidant activity of the benzimidazole core.

REFERENCES

1. C. Mallappaji, S. S. Jagadish, T. R. Swaroop, R. Roopashree, C. D. Mohan, K. S. Rakesh, B. Raghava and K. S. Rangappa, *Asian J. of Biochem. Phar. Research*, **2015**, *4*, 71-81.
2. K. G. Desai and K. R. Desai, *Bioorg. Med. Chem*., **2006**, *14*, 8271-8279.
3. I. Tamm, M. M. Nemes and S. Osterhout, *J. Exp. Med*., **1960**, *111*, 339-349.
4. W. G. Schulz, I. Islam and E. B. Skibo, *J. Med. Chem*., **1995**, *38*, 109-118.
5. S. Grivennikov, F. R. Greten and M. Karin, *Cell*, **2010**, *140*, 883-899.
6. C. Kus, G. A. Kılcıgil, S. Ozbey, F. Betu, I. Kaynak, M. Kaya, T. Coban and B. C. Eke, *Bioorg. Med. Chem.*, **2008**, *16*, 4294-4303.
7. N. H. Haul, H. Nar, H. Priepke, U. Ries, J. M. Stassen and W. Wienen, *J. Med. Chem.,* **2002**, *45*, 1757-1766.
8. R. M. Kim, J. Chang, A. R. Lins, E. Brady, M. R. Candelore, Q. D.Yang, V. Ding, J. Dragovic, S. Iliff, G. Jiang, S. Mock, S. Qureshi, R. Saperstein, D. Szalkowski, C. Tamvakopoulos, L. Tota, M. Wright, X. Yang, J. R. Tata, K. Chapman, B. B. Zhang, E. R. Parmee, *Bio. & Med. Chem. Lett*., **2008**, *18,* 3701-3705.
9. K. L. Erb, S. B. Ravula, J. Yu, S. Z. Kord, W. J. Moree, R. E. Petroski, J. Wen, S. Malany, S. R. J. Hoare, A. Madan, P. D. Crowe and G. Beaton, *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 2916-2919.
10. B. Yogita and S. Om, *Bioorganic & Med. Chem*., **2012**, *20*, 6208-6236.
11. N. Karaali and E. Menteşe, *Rev. Roum. Chimie*, **2016**, *61*, 187-192.
12. E. Menteşe, N. Karaali, F. Yilmaz, S. Ülker and B. Kahveci, *Archiv der Pharmazie*, 2013, **346**, 556-561.
13. E. Menteşe, F. Yilmaz, N. Baltaş, O. Bekircan and B. Kahveci, *J. Enzyme Inhib. and Med. Chem.,* **2015**, *30*, 435-441.
14. R. Apak, K. Guclu, M. Ozyurek and S. E. Karademir, *J. Agr. Food Chem.,* **2004**, *52*, 7970-7981.
15. F. Yilmaz, N. Karaali and S. Sasmaz, *Bull. Chem. Soc. Ethiopia*, **2017**, *31*, 351-359.
16. F. Yilmaz, E. Menteşe and N. Baltaş, *Letters in Drug Design & Discovery*, **2017**, *14*, 201-208.
17. E. Wyrzykiewicz and D. Prukah, *J. Heterocycl. Chem.,* **1998**, *35*, 381-387.
18. A. Usta, F. Yılmaz, G. Kapucu, N. Baltaş and E. Menteşe, *Lett. Org. Chem.,* **2015**, *12*, 227-232.
19. B. Kahveci, F. Yılmaz, E. Menteşe, M. Özil and S. A. Karaoğlu, *J. Heterocycl. Chem.,* **2014***, 51*, 982-990.
20. E. Mentese, N. Karaali, F. Yilmaz, S. Ulker and B. Kahveci, *Arch. Pharm.,* **2013**, *346,* 556-561.
21. N. Baltaş, F. Yılmaz and E. Menteşe, *Hacettepe J. Biol. & Chem.,* **2016***, 44*, 293-305.
22. O. Bekircan, N. Baltaş, E. Menteşe and E. Gültekin, *Rev. Roum. Chim.*, **2016**, *61*, 733-746.
23. W. Brandwilliams, M. E. Cuvelier and C. Berset, *Food Sci. Technol-Leb*., **1995**, *28*, 25-30.
24. G. K. Kantar, N. Baltaş, E. Menteşe and S. Şaşmaz, *J. Organomet. Chem.*, **2015**, *787*, 8-13.
25. C. Kantar, V. Mavi, N. Baltas, F. Islamoglu and S. Sasmaz, *J. Molec. Structure,* **2016***, 1122*, 88-99.
26. M. W. Weatherburn, *Anal. Chem.,* **1967**, *39*, 971-974.