Synthesis, Antioxidant ability and Docking study for new 4,4'-((2-(Aryl)-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(methylene))diphenol)

Raied Mustafa Shakir[1]*, Shaimaa Abed Saoud[1], Dhuha Faruk Hussain[1], Khalid Fahad Ali[1], Firas Shawqi Algburi[2] and Husam Salman Jasim[1]

1. Department of Chemistry, Ibn Al-Haitham, University of Baghdad, Baghdad 61023, IRAQ
2. Department of Biology, College of Science, Tikrit University, IRAQ
*raiedalsayab@yahoo.co.uk

Abstract

New series of 4,4'-((2-(Aryl)-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(methylene))Diphenol(3a-g) was successfully synthesized from cyclization of the reduction product of bis Schiff bases (2) with aryl aldehydes bearing phenolic hydroxyl in the presence of acetic acid. The structure of these compounds was identified from FT-IR, ^{1}H NMR, ^{13}C NMR and EIMs. The Antioxidant capability was screened by DPPH and FRAP assays. Both assays showed antioxidant capability more than BHT as well. Compounds 3b and 3c showed antioxidant capacity slightly less than ascorbic acid. The docking study for theses compound was carried out as III DNA polymerase inhibitor.

The results of docking demonstrated that the increase in hinderances around phenolic hydroxyl for the aryl attached position two for benzimidazole decrease the capability of interaction and give less bending and smaller docking score and there is inverse relationship between increasing hindrances around phenolic hydroxyl and DNA polymerase inhibition for these compounds.

Keywords; Benzimidazole, Diphenol, Antioxidant, DPPH, FRAP, DNA polymerase.

Introduction

Benzimidazole is gaining vast importance in synthetic organic chemistry due to its pharmacological uses. Medicines containing benzimidazole group in their structure are wildly known. For example, albendazole and mebendazole are benzimidazole anthelmintics used to treat helminth infections and parenchymal neurocysticercosis[15]. Researchers are paying considerable attention to benzimidazole derivatives. These derivatives also exhibited various biological activities[26]. Benzimidazole containing 1,3,4-oxadiazole displays anticancer activity[13], moreover palladium allyl derivative of benzimidazole shows anticancer activity[15]. These various activities can be summarized such as antifungal[6,14], antituberculosis agents[17], antibacterial[2,4], anti-inflammatory[9], anti-biofilm and anti-microbial agents[1], analgesic[7], anti-HIV[5,11] and antioxidant[3,28]. The damages in bio cells from free radicals are wide recognized.[21,27]

Furthermore, antioxidants compounds are gaining interest due to their ability to prohibit propagation reaction of free radical or prevent formation of free radical. Phenolic compounds such as hindered phenolic or polyphenols, flavonoids and multi phenols compounds are one of the renowned antioxidants[18,19,23]. The phenolic compounds besides to their antioxidant ability exhibited diverse biological activities likewise antidiabetic, anti-inflammatory and anticancer agents[25], anti-HIV[8], antiallergic[10] and anti-Parkinson's disease[29]. In this work, new benzimidazole derivatives were synthesized containing multi phenolic group. The antioxidant activity of these compounds was screened by utilizing DPPH and FRAP assays as well the molecular structure docking achieved as inhibiter of III DNA polymerase which is extremely significant objective molecules of antitumor factor.[12]

Material and Methods

Synthesis of 4,4'-(1,2-phenylenebis(azaneylylidene))bis (methaneylylidene))diphenol 1: This product was synthesized from modification procedure described by Rao et al.[20] A solution of 1,2-phenylenediamine (2 g, 0.01 mmol) in 15 mL absolute ethanol was added in small portions to a stirring solution of 4-hydroxybenzaldehyde (2.26 g, 0.01 mmol) in 15 mL absolute ethanol with three drops of acetic acid. The mixture was heated under reflux for 5 hrs. Then it was cooled to room temperature, the precipitated was collected and washed with cold ethanol and then dried. Recrystallized by ethanol to give pale yellow precipitate. Yield 91 %, MP. 194-197℃ (lit 196-197)[20]. FT IR (KBr, υ_{max}/ cm^{-1}); 3456 (OH), 3085 (CH_{Ar}), 1633 (C=N), 1597(C=C), ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 6.78 (d, 4H, *J* 8.22 Hz, Ar-H), 7.24-7.38 (m, 4H, Ar-H), 7.75 (d, 4H, *J* 8.6 Hz, Ar-H), 8.56 (s, 2H, 2×C=N), 10.53 (bs, 2OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 119.11, (4C), 123.08 (2C), 125.45 (2C), 128.32 (2C), 130.53 (4C), 130.74 (2C), 152.09 (2C, 2×C=N), 156.15 (2C). EIMS found: 316 [$M^{\bullet+}$] (calc. for $C_{20}H_{16}N_2O_2$, $C_{20}H_{16}N_2O_2$, 316.12).

Synthesis of 4,4'-((1,2-phenylenebis(azanediyl))bis (methylene))diphenol. 2: This compound was synthesized according to described procedure.[24] Small portions of sodium borohydride (0.6 g, 0.01 mmol) were added to a stirring solution of compound 1 in 15 mL of methanol-tetrahydrofurane (1:1). After completion, 20 mL of crushed ice was added and the mixture stirred vigorously for half hour. The precipitate was collected by filtration and recrystallized after drying from aqueous methanol to give

white needle crystals: Yield 83 %, MP 218-220 ℃; FT IR (KBr, U_{max}/ cm^{-1}); 3398 (OH), 3322 (NH), 3061 (CH_{Ar}), 2987, 2846 ($CH_{aliphatic}$), 1594 (C=C), ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 4.42 (s, 4H, $2CH_2$), 6.51-6.73 (m, 10H, Ar-H and 2NH), 7.16 (4H, d, *J* 8.62 Hz, ArH). ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 46.83 (2C, $2CH_2$), 117.09 (4C), 117.88 (2C), 120.71 (2C), 131.18 (4C), 131.79 (2C), 133.23 (2C), 157.04 (2C); EIMS found: 320 [$M^{\bullet+}$] (calc. for $C_{20}H_{20}N_2O_2$, 320.15).

General synthesis of 4,4'-((2-(Aryl)-1H-benzo[d] imidazole-1,3(2H)-diyl)bis(methylene))

Diphenol (3a-g): An aryl aldehyde (0.01 mmol) and 4,4'-((1,2-phenylenebis (azanediyl))bis(methylene))diphenol were dissolved in acetic acid (15-25 mL) and heated under reflux for 7-9 hrs. Upon cooling to room temperature, the mixture was acerbated on refrigerator overnight.

The precipitate was collected and washed with cold water (3 × 20 mL). The crude compounds were purified either from column chromatography or recrystallized from suitable solvent.

4,4'-((2-(4-hydroxyphenyl)-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(methylene))diphenol 3a: The crude precipitate was purified by column chromatography using eluent from diethyl ether: methanol (9:1) to obtain white amorphous substance: Yield 74 %, MP 229-230 ℃; FT IR (KBr, U_{max}/ cm^{-1}); 3420 (OH), 3069 (CH_{Ar}), 2956, 2877 ($CH_{aliphatic}$), 1600 (C=C), ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 4.62 (s, 4H, $2CH_2$), 5.52 (s, 1H, CH), 6.58-6.77 (m, 6H, Ar-H), 7.01-7.03 (d, 4H, *J* 8.2 Hz, Ar-H), 7.31(d, 2H, *J* 8.4 Hz, Ar-H), 7.44-7.62 (m, 4H, Ar-H), 9.11 (bs, 2H, 2×OH), 9.47 (bs, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 44.49 (2C, $2CH_2$), 83.67 (1C, CH) ,112.22 (2C), 117.09 (4C), 117.56 (2C), 120.11 (2C), 130.51 (2C), 132.08 (2C), 132.45 (4C), 133.72 (1C), 143.27 (2C), 154.34 (1C), 155.53 (2C); EIMS found: 424 [M˙+] (calc. for $C_{27}H_{24}N_2O_3$, 424.18).

4,4'-((2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1H-benzo[d] imidazole-1,3(2H)-diyl)bis(methylene))diphenol. 3b: The resulting precipitate was recrystallized from DCM to obtain white precipitate: Yield 78 %, M.P. 230-232 ℃, FT IR (KBr, U_{max}/ cm^{-1}); 3462 (OH), 3074 (CH_{Ar}), 2971, 2862 ($CH_{aliphatic}$), 1597 (C=C), ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.41 (s, 18H, $C(CH_3)_3$), 4.57 (s, 4H, $2×CH_2$), 5.62 (s, 1H, OH) 5.74 (s, 1H, CH), 6.51-6.73 (m, 8H, Ar-H), 7.44 (d, 4H, *J* 8.46, Ar-H), 7.81 (s, 2H, Ar-H), 9.24 (bs, 2H, 2×OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 29.93 (6C, $2C(CH_3)_3$), 34.41 (2C, $2C(CH^3)^3$), 45.92 (2C, $2CH_2$), 82.97 (1C, CH), 110.8 (2C), 116.06 (4C), 120.21 (2C), 126.12 (2C), 130.32 (1C), 131.43 (4C), 133.77 (2C), 138.35 (2C), 144.03 (2C), 154.83(1C); EIMS found: 536 [M˙+] (calc. for $C_{35}H_{40}N_2O_3$, 536.30).

4,4'-((2-(3,5-di-*tert*-butyl-2-hydroxyphenyl)-1H-benzo[d] imidazole-1,3(2H)-diyl)bis(methylene)) diphenol. 3c: The product was recrystallized by acetonitrile to obtain white amorphous substance: Yield 68 %, M.P. 227-229 ℃; FT IR (KBr, U_{max}/ cm^{-1}); 3412 (OH), 30032 (CH_{Ar}), 2961, 2847 ($CH_{aliphatic}$),1589 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.28 (s, 9H, $3×CH_3$), 1.34 (s, 9H, 3×CH3), 5.03 (s, 4H, $2×CH_2$), 5.97 (s, 1H, CH), 6.57-6.73 (m, 8H, Ar-H), 6.97 (d, 4H, *J* 8.24 Hz, Ar-H), 7.11 (d, 1H, *J* 2.22 Hz, Ar-H), 7.30 (d, 1H, *J* 2.24 Hz , Ar-H), 9.15 (bs, 1H, OH), 9.88 (bs, 2H, 2×OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 29.83 (3C), 31.67 (3C), 34.46 (1C), 35.87 (1C), 48.22 (2C), 79.19 (1C, CH), 107.35 (2C), 108.42(1C), 109.73 (2C), 113.41 (4C), 12.49 (1C), 128.33 (1C), 130.81 (2C), 132.19 (4C), 137.05 (1C), 140.98 (1C), 142.87 (2C), 151.23 (1C), 156.11 (2C); EIMS found: 536 [$M^{\bullet+}$] (calc. for $C_{35}H_{40}N_2O_3$, 536.30).

4,4'-((2-(4-hydroxy-3,5-dimethoxyphenyl)-1H-benzo[d] imidazole-1,3(2H)-diyl)bis(methylene))diphenol 3d: The crude precipitate was recrystallized from chloroform to give white crystals: Yield 66%, MP 234-236 ℃; FT IR (KBr, U_{max}/ cm^{-1}); 3431 (OH), 3061 (CH_{Ar}), 2962, 2877 ($CH_{aliphatic}$), 1598 (C=C), 1217 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 3.81 (s, 6H, OCH_3), 4.76 (s, 4H, $2×CH_2$), 6.01 (s, 1H, CH), 6.46-6.63 (m, 6H, Ar-H), 6.85 (d, 4H, *J* 8.8 Hz,Ar-H,), 7.19 (d, 4H, *J* 8.84 Hz, Ar-H), 9.18 (bs, 1H, OH), 9.58 (bs, 2H, 2×OH).; ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 49.44 (2C), 55.83 (2C), 78.39 (1C), 108.41 (2C), 111.51 (2C), 116.32 (4C), 129.07 (2C), 131.35 (2C), 132.57 (4C), 134.12 (1C), 139.88 (1C), 142.78 (2C), 153.66 (2C) 154.81(2C); EIMS found: 484 [$M^{\bullet+}$] (calc. for C29H28N2O5 , 484.20).

4,4'-((2-(2-hydroxy-3-methoxyphenyl)-1H-benzo[d] imidazole-1,3(2H)-diyl)bis(methylene))diphenol 3e: The resulting product was recrystallized from ethanol to give shiny pale yellow crystals: Yield 54% ,MP 233-235 ℃; FT IR (KBr, U_{max}/ cm^{-1}); 3554 (OH), 3052 (CH_{Ar}), 2971,2867($CH_{aliphatic}$), 1596 (C=C), 1211 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 3.85 (s, 3H, OCH_3), 4.64 (s, 4H, CH_2), 6.25 (s, 1H, CH), 6.42-6.83 (m, 11H, Ar-H), 7.26 (d, 4H, *J* 8.2 Hz, Ar-H), 9.18 (bs, 2H, OH), 9.77 (bs, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) ppm;49.82 (2C), 75.75 (1C), 110.17 (1C), 111.58 (2C), 115.87 (4C), 119.19 (2C), 120.11 (1C), 121.24 (1C), 122.07 (1C), 122.49 (1C), 131.57 (4C), 143.20 (2C), 150.76 (1C), 152.32 (1C), 154.19 (2C); EIMS found: 454 [$M^{\bullet+}$] (calc. for $C_{28}H_{26}N_2O_4$, 454.19).

4,4'-((2-(2-hydroxy-3-methylphenyl)-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(methylene))diphenol 3f: The resulting product was recrystallized from acetonitrile to obtain white amorphous powder: Yield 58%, MP 270 ^{0}C; FT IR (KBr, U_{max}/ cm^{-1}); 3537 (OH), 3072 (CH_{Ar}), 2934,2879 ($CH_{aliphatic}$), 1597 (C=C), 1224 (C-O-C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.26 (s, 3H, CH_3), 5.21 (s, 4H, $2xCH_2$), 6.19 (s, 1H, CH) 6.45-6.79 (m, 8H, Ar-H), 7.19-7.30 (m, 7H, Ar-H), 9.26 (bs, 1H, OH), 9.79 (bs, 2H, OH).^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm;21.13 (1C), 48.27 (2C), 76.09 (1C), 111.68 (2C), 115.77 (4C), 119.64 (2C), 120.32 (1C), 121.48 (1C), 126.35 (1C), 127.51 (1C), 130.17 (1C), 132.42 (4C), 142.92 (2C), 152.13 (1C), 155.01

(2C); EIMS found: 438 [$M^{\bullet+}$] (calc. for $C_{28}H_{26}N_2O_3$, 438.19).

4,4'-((2-(2-hydroxyphenyl)-1H-benzo[d]imidazole-1,3(2 H)-diyl)bis(methylene))diphenol 3g: The product was purified by column chromatography utilizing hexane-ethyl acetate (5-1) as mobile phase to afford white precipitate: Yield 56%, MP 176-179 ℃;FT IR (KBr, U_{max}/ cm^{-1}); 3545 (OH), 3037 (CH_{Ar}), 2978,2862 ($CH_{aliphatic}$),1595 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 4.85 (s, 4H, $2CH_2$), 5.97 (s,1H, CH), 6.53-7.09 (m, 15H, Ar-H), 7.37 (d, 1H, *J* 8.2 Hz, Ar-H), 9.38 (bs, 2H, 2×OH), 10.03 (bs, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 48.12 (2C), 76.56 (1C), 112.51 (2C), 116.82 (4C), 117.07 (1C), 120.12 (2C), 120.98 (1C), 122.72 (1C), 128.84 (1C), 129.46 (1C), 132.28 (4C), 143.15 (2C), 152.91 (1C), 154.76 (2C); EIMS found: 424 [$M^{\bullet+}$] (calc. for $C_{27}H_{24}N_2O_3$, 424.18).

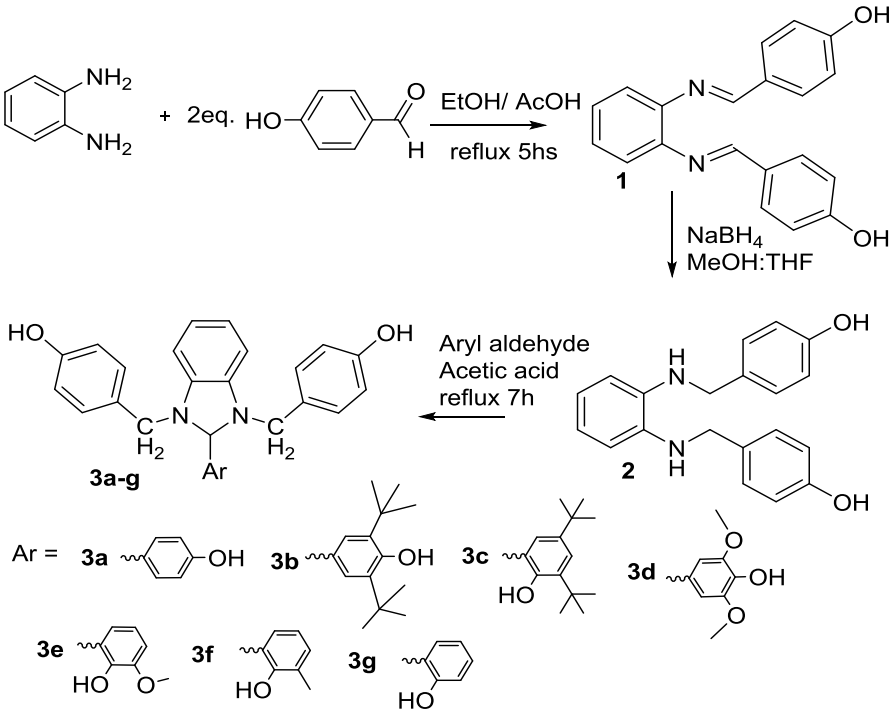
Results and Discussion

The series of 4,4'-((2-(Aryl)-1H-benzo[d]imidazole-1,3 (2H)-diyl)bis(methylene))diphenol was synthesized by three steps. The first step was synthesis of bis Schiff bases (1) from reacting equivalents millimole of 4-hydroxybenzaldehyde with 1,2-phenylenediamine. The next step was reduction of the bis Schiff bases by sodium borohydride to produce compound (2). The final step by cyclization reaction of compound (2) with substituted benzaldehyde in concentration acetic acid to produce (3a-g) as demonstrated in scheme 1.

The FT-IR of compound (1) displayed C=N band at 1633 cm^{-1}. The broad band of phenolic hydroxyl was assigned at 3456 cm^{-1}. The FT-IR of compound (2) displayed new band of NH assigned at 3322 cm^{-1}. Furthermore, the FT-IR spectrum showed the $CH_{aliphatic}$ at (2987,2846) cm^{-1}. While, the band of NH group vanished from FT-IR spectra of compounds (3a-g). ^{1}H NMR of compound (1) showed the peak of imine group at δ 8.56 ppm and it disappeared from ^{1}H NMR of compound (2) and new peak with integral four proton appeared at δ 4.42 ppm for two CH_2 group.

The ^{1}H NMR of compounds (3a-g) showed disappearance of two protons of NH which was considered first evidence of successful cyclization. Furthermore, new peak appeared at range δ 5.52-6.25 ppm with integral of one proton for CH of benzimidazole ring and the two CH_2 of benzyl groups appearing at δ 4.57-5.21 ppm and the two phenolic hydroxyl of 4-hydroxybenzyl assigned at δ 9.11-9.88 ppm. The protons of aryl group at position two of benzimidazole ring and their substituted group appeared at their proposed range. The ^{1}H NMR of compound (3a) showed integration of aromatic system equal to twelve protons besides three protons of three phenolic hydroxyl. The spectrum of (3b) displayed the protons of two di tert butyl groups at δ 1.41 ppm with integral 18 H, as well the hindered phenolic hydroxyl appeared at δ 5.62 ppm.

The spectrum of (3c) showed protons of two tert butyl group at δ 1.28 ppm and δ 1.34 ppm with integral 9 H for each peak and the proton of phenolic hydroxyl of δ 9.88 ppm di-tert-butyl located at 9.15 ppm. The ^{1}H-NMR spectrum of (3d) shows two protons of syringyl, nine protons for two groups of methoxy located at δ 3.81 ppm. Moreover, the hydroxyl group of siringyl was located at δ 9.18 ppm. The spectra of (3e) demonstrated three protons of methoxy group at δ 3.85 ppm and all aromatic peaks at hydroxyl group of the aryl at position two was located at δ 9.77 ppm. The ^{1}H NMR of (3f) shows all expected protons.



Scheme 1: Pathway of Synthesis of 3a-g

The methyl group was located at δ 2.26 ppm as well the hydroxyl group of the aryl was located at δ 9.26 ppm besides the aromatic protons. Spectrum of (3g) showed the protons of salicyl group at their predicted regions besides their hydroxyl peaks located at δ 10.03 ppm.

The ^{13}C NMR of compounds (3a-g) confirmed their structure and successful cyclization. The identical carbon for C2 of benzimidazole ring was located at δ 75.75-83.67 ppm besides to the carbon attached with phenolic hydroxyl which is assigned at downfield of the spectrum. Furthermore, all carbons of phenolic group were attached with this carbon and their substituted groups located at their proposed region. For instance, the spectrum of compound (3b) shows two peaks for two groups of di-tert-butyl at δ 29.93 ppm for $C(CH_3)_3$ besides to δ 34.41 ppm for $C(CH_3)_3$, while compound (3c) displayed four peaks at δ (29.83, 31.67, 34.46, 35.87) ppm for two group of tert-butyl at position 3 and 5.

The two methoxy groups of compound (3d) were situated at δ 55.83 ppm and the methoxy group of compound (3e) was situated at δ 75.75 ppm. The methyl group for compound (3f) was situated at δ 21.13 ppm. The mass spectra harmonized with FT-IR,^{1}H-NMR ^{13}C NMR confirm the structure. The mass spectra for these compounds displayed the ionic mass and the base peaks as well the fragments corresponding to the structures of compound (3a-g).

Antioxidant screening: The antioxidant activity for the synthesized compound (3a-g) was tested by DPPH and FRAP assays. Both assays were screened according to procedures described by Saoud et al.[22] In DPPH assay, ascorbic acid and BHT were utilized as refences. The DDPH inhibition displayed that the compound 3b possessed highest antioxidant activity in all synthesized compounds. This activity is slightly less than ascorbic acid inhibition percentage, moreover it possesses IC_{50} ((half maximal scavenging concentration) slightly higher than ascorbic acid. Compound 3c possessed next highest antioxidant activity as shown in table 2.

The sequence of free radical scavenging for these synthesized compounds exhibited that the phenolic ring attached to carbon at position two of benzimidazole ring plays important role to enhance the antioxidation properties. The two groups of 4-hidroxybenzyl possess a minor impact in antioxidant activity for these compounds. On the other hand, comparing the DPPH inhibition between 3a and 3g indicted that phenolic hydroxyl at position ortho is more effective. The benzimidazole ring has good contribution to increase hinderance around phenolic hydroxyl at position ortho. All synthesized compounds showed antioxidant capacity more than BHT. The FRAP value for these compounds (Figure 1) was in agreement with DPPH inhibition percentage and demonstrated same sequence of antioxidant properties for these compounds.

Table 1
Selective physical properties of the synthesized compounds (1-3a-g).

Compound No.	Yield %	M.P ℃	Fw	EIMs calc.	EIMs found
3a	74	229-230	$C_{27}H_{24}N_2O_3$	424.18	424
3b	78	230-232	$C_{35}H_{40}N_2O_3$	536.30	536
3c	68	227-229	$C_{35}H_{40}N_2O_3$	536.30	536
3d	66	234-236	$C_{29}H_{28}N_2O_5$	484.20	484
3e	54	233-235	$C_{28}H_{26}N_2O_4$	454.19	454
3f	58	270 dec.	$C_{28}H_{26}N_2O_3$	438.19	438
3g	56	176-179	$C_{27}H_{24}N_2O_3$	424.18	424

Table 2
DPPH and IC_{50} of synthesis compounds 3a-g

Compound No.	DPPH Inhibition % ± SD[a]	IC_{50} ± SEM [b] (100 µg/mL)
3a	67.39±0.061	77.05±0.048
3b	88.27±0.043	22.98±0.028
3c	83.19±0.059	28.39±0.016
3d	78.36 ±0.035	62.22±0.038
3e	74.12±0.054	70.19±0.066
3f	73.79±0.072	73.71±0.041
3g	71.57±0.029	75.37±0.072
BHT	66.83±0.058	78.27±0.036
Ascorbic acid	90.67± 0.0.32	23.18±0.041

[a] (SD) Standard Deviation; [b] (SEM) Standard Error of the Mean and IC50: 50% effective concentration

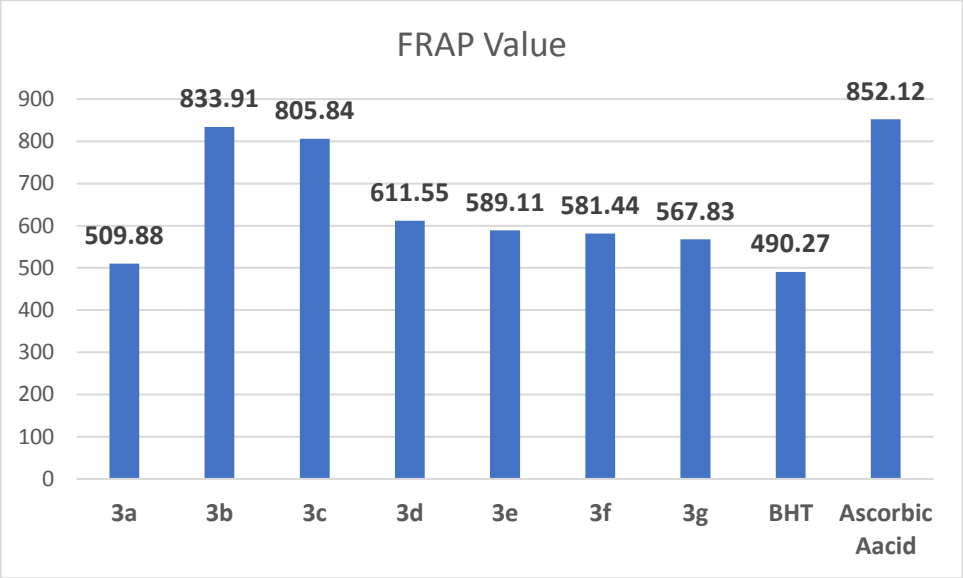


Figure 1: FRAP value for newly synthesized compounds 3a-g

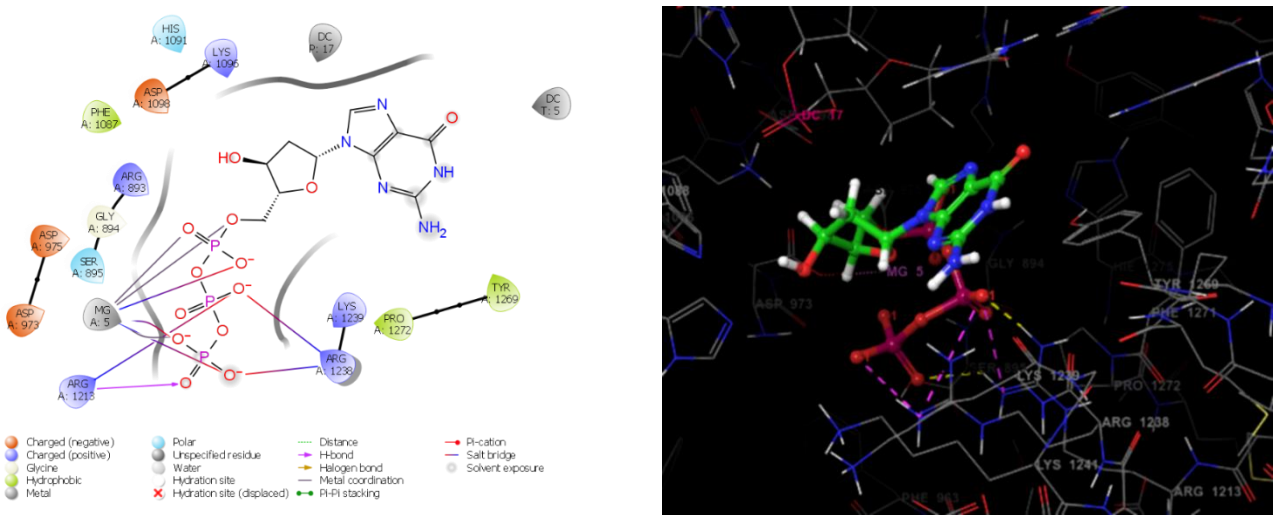


Figure 2: Exhibiting binding mode of natural nucleotide

Table 3
RMSD (Root Mean Square Deviation), the docking scores, type of banding and their distance of standard

Compound No.	RMSD	Docking Score	Type of bond	Distance Aº
Standard	0.039	-8.073	H-bond	2.6
			Salt bridg	3.67
			Salt bridg	3.73
			Salt bridg	3.84
			Salt bridg	4.19
			Salt bridg	4.62
			Metal coordination	2.01
			Metal coordination	2.06

Docking study: All compounds were installed at binding sites for III DNA polymerase with Schrödinger Maestro version 12.5.139 and MMshare 5.1.139 version used for all docking simulations. From the results of the analysis of the fusion between the prepared compounds and the DNA replication enzyme, insights were obtained about the interactions between the prepared compounds and the enzyme, as well as the use of the natural nucleotide as a reference for the prepared compounds. Figure 2 depicted bending mode of nucleotide. RMSD (Root Mean Square Deviation), the docking scores, type of banding and their distance of standard were tabulated in table 3.

The docking study for compound 2 as inhibitor of III DNA polymerase showed highest negative value of docking score (-8.928) with RMSD equal to 0.047 (Figure 3).

The simulation study of compound 3a displayed six bonds, as two bonds as H-bond. pi-bond and metal coordination with RMSD equal to 0.039 and docking score equal to 8.073.

The bonds length of all synthesized compounds (3a-g) is listed in table 4. Compound 3b displayed three bonds, two as H-bond and one bond as metal coordination with RMSD equals 0.045 and docking score to 4.462. Figure 4b depicted the binding mode of these compounds.

Compound 3c exhibited only three hydrogen bonding with RMSD and docking score equal to 0.037 and -7.111 respectively whereas compound 3d exhibited just two pi - bond and two metal coordination as depicted in figure 5.

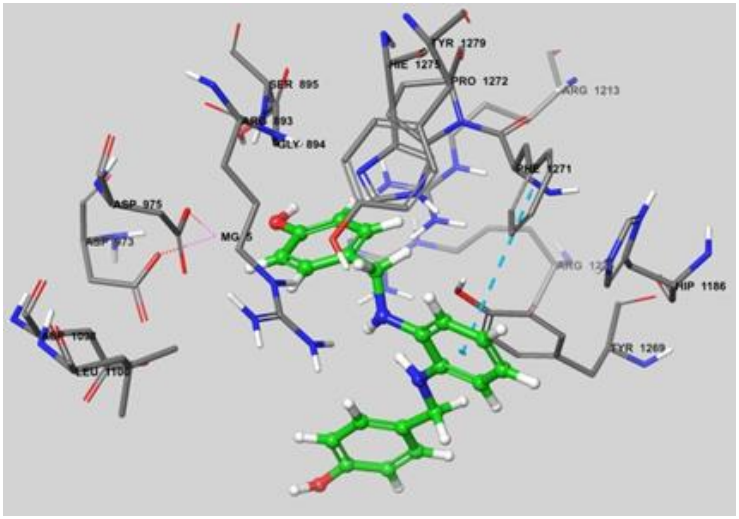
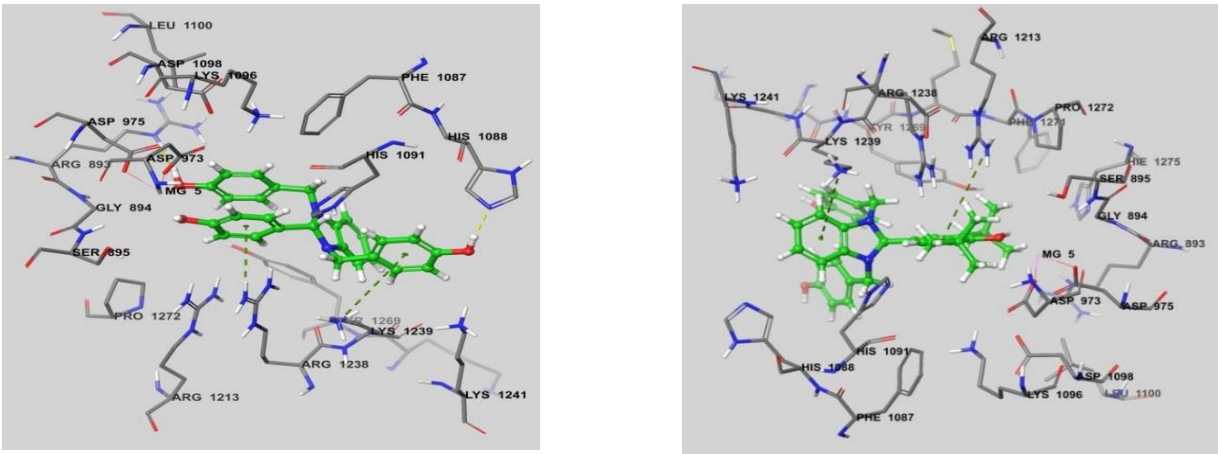
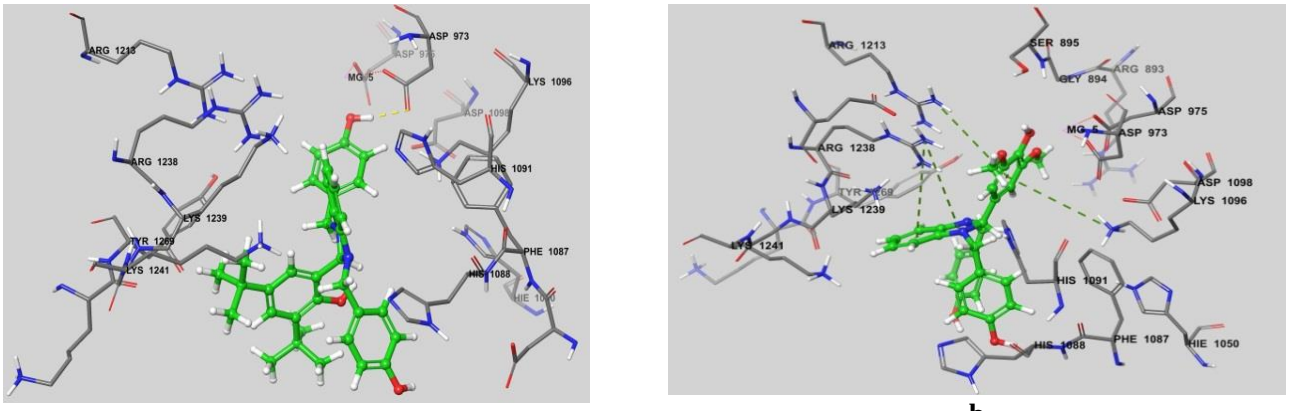


Figure 3: Exhibiting binding mode of compound 2



a **b**

Figure 4: Binding mode of compounds 3a (a) and 3b (b) as inhibiter of III DNA polymerase

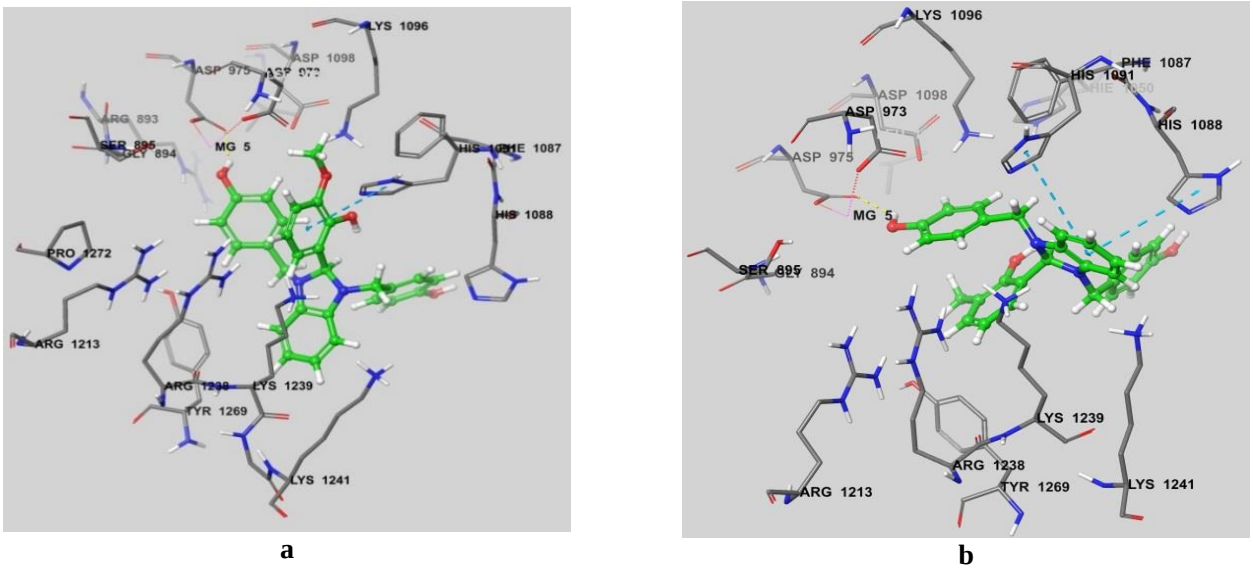


a **b**

Figure 5: Binding mode of compounds 3c (a) and 3d (b) as inhibiter of III DNA polymerase

Table 4
Parameters of docking study for synthesized compound 2-(3a-g)

Compound No.	RMSD	Docking Score	Type of bond	Distance Aº
2	0.047	-8.928	H-bond	1.57
			Pi-Pi Stacking	5.40
			Metal coordination	2.07
3a	0.047	-8.098	H-bond	1.95
			H-bond	1.61
			Metal coordination	2.08
			Metal coordination	2.13
			Pi-Cation	4.23
			Pi-Cation	5.18
3b	0.045	-4.462	Pi-Cation	5.87
			Pi-Cation	6.02
			Metal coordination	2.36
3c	0.037	-7.111	H-bond	1.69
			H-bond	1.98
			H-bond	2.19
3d	0.046	-6.610	Pi-Cation	5.76
			Pi-Cation	6.15
			Metal coordination	2.06
			Metal coordination	2.13
3e	0.046	-7.454	H-bond	1.80
			H-bond	1.86
			H-bond	1.95
			Pi-Pi Stacking	4.83
			Metal coordination	2.01
3f	0.042	-7.326	H-bond	1.79
			H-bond	1.91
			H-bond	2.19
			Pi-Pi Stacking	5.08
			Pi-Pi Stacking	5.18
			Metal coordination	2.01
3g	0.047	-7.252	H-bond	1.87
			Metal coordination	2.04



a **b**

Figure 5: Binding mode of compounds 3e(a) and 3f(b)

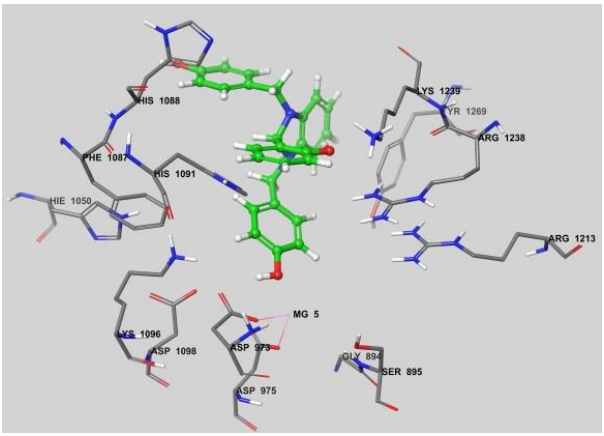


Figure 6: Binding mode of compounds 3g

Compound 3e exhibited five bonding, three of them as hydrogen bonding as Pi-Pi stacking and the last one as metal coordination and it showed RMSD equal to 0.046 with docking score equal to -7.454. The docking study of compound 3f exhibited six bonding, three of them as hydrogen bonding and two as Pi - Pi stacking and the last bonding as metal coordination. The simulation study for compound 3g exhibited just two bonding- one as hydrogen bonding and the other one as metal coordination as depicted in figure 6.

These results demonstrated that compound 2 possesses just three bonding, it has the highest docking score. For the compounds 3a-g, the docking exhibited that increasing steric hindrances around the phenolic group attached in position two off benzimidazole decrease their capability of interaction and give less docking scores value as in compound 3b. Furthermore, the results show that the compounds possess steric hindrances around phenolic hydroxyl at position ortho exhibited interaction capability preferable than those have phenolic hydroxyl at position para hydroxyl.

Conclusion

Newly series of 4,4'-((2-(Aryl)-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(methylene))Diphenol was successfully synthesized from cyclization. The structure of these compounds was confirmed by FT-IR, 1D NMR and EIMs. The anti-oxidant capability for these compounds was tested by DPPH and FRAP assays. All these compounds exhibited antioxidant capacity more than BHT and compounds 3d and 3c displayed significant antioxidant capacity slightly less than ascorbic acid. The antioxidant screening showed that the phenolic aryl at position two can play important role for enhancing the antioxidant ability.

The docking study for these compound as III DNA polymerase inhibitor showed that the increased hinderances around phenolic hydroxyl for the aryl at position two for benzimidazole decrease the capability of interaction and give less bending and smaller docking score and there is inverse relationship between increasing hindrances around phenolic hydroxyl and DNA polymerase inhibition.

Acknowledgement

The authors would like to acknowledge University of Baghdad and College of Education for pure science-Ibn Al-Haitham for supporting this work.

References

1. Abraham R., Prakash P., Mahendran K. and Ramanathan M., A novel series of N-acyl substituted indole-linked benzimidazoles and naphthoimidazoles as potential anti inflammatory, anti biofilm and anti microbial agents, *Microbial Pathogenesis*, **114**, 409 **(2018)**

2. Al-Mohammed N.N., Alias Y., Abdullah Z., Shakir R.M., Taha E.M. and Hamid A.A., Synthesis and antibacterial evaluation of some novel imidazole and benzimidazole sulfonamides, *Molecules*, **18(10)**, 11978 **(2013)**

3. Aroua L.M., Almuhaylan H.R., Alminderej F.M., Messaoudi S., Chigurupati S., Al-mahmoud S. and Mohammed H.A., A facile approach synthesis of benzoylaryl benzimidazole as potential α-amylase and α-glucosidase inhibitor with antioxidant activity, *Bioorg Chem*, **114**, 105073 **(2021)**

4. Baron A., Sann C.L. and Mann J., Symmetric bis-benzimidazoles as DNA minor groove-binding agents with anti-tumour and antibacterial activity and the evolution of the drug ridinilazole for the treatment of CLOSTRIDIUM difficile infections, *Biorg Med Chem*, **58**, 116656 **(2022)**

5. Bi X., Meng X., Chen G., Chen B. and Zhao P., Manganese oxide catalyzed synthesis of anti-HIV N-substituted benzimidazoles via a one-pot multistep process, *Catal Commun*, **116**, 27 **(2018)**

6. Çevik U.A., Celik I., Işık A., Pillai R.R., Tallei T.E., Yadav R., Özkay Y. and Kaplancıklı Z.A., Synthesis, molecular modeling, quantum mechanical calculations and ADME estimation studies of benzimidazole-oxadiazole derivatives as potent antifungal agents, *J Mol Struct*, **1252**, 132095 **(2022)**

7. Gaba M., Singh S. and Mohan C., Benzimidazole: An emerging scaffold for analgesic and anti-inflammatory agents, *European Journal of Medicinal Chemistry*, **76**, 494 **(2014)**

8. Hassan Khan M.T. and Ather A., Potentials of phenolic molecules of natural origin and their derivatives as anti-HIV

agents, In Biotechnology Annual Review, edited by El-Gewely MRElsevier, 223 **(2007)**

9. Maghraby M.T.E., Abou-Ghadir O.M.F., Abdel-Moty S.G., Ali A.Y. and Salem O.I.A., Novel class of benzimidazole-thiazole hybrids: The privileged scaffolds of potent anti-inflammatory activity with dual inhibition of cyclooxygenase and 15-lipoxygenase enzymes, *Biorg Med Chem*, **28(7)**, 115403 **(2020)**

10. Medeiros K.C.P., Figueiredo C.A.V., Figueredo T.B., Freire K.R.L., Santos F.A.R., Alcantara-Neves N.M., Silva T.M.S. and Piuvezam M.R., Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice, *J Ethnopharmacol*, **119(1)**, 41 **(2008)**

11. Miller J.F., Turner E.M., Gudmundsson K.S., Jenkinson S., Spaltenstein A., Thomson M. and Wheelan P., Novel N-substituted benzimidazole CXCR4 antagonists as potential anti-HIV agents, *Bioorg Med Chem Lett*, **20(7)**, 2125 **(2010)**

12. Miura S. and Izuta S., DNA Polymerases as Targets of Anticancer Nucleosides, *Current Drug Targets*, **5(2)**, 191 **(2004)**

13. Mochona B., Mazzio E., Gangapurum M., Mateeva N. and Redda K.K., Synthesis of Some Benzimidazole Derivatives Bearing 1,3,4-Oxadiazole Moiety as Anticancer Agents, *Chemical Science Transactions*, **4(2)**, 534 **(2015)**

14. Morcoss M.M., Abdelhafez E.S.M.N., Ibrahem R.A., Abdel-Rahman H.M., Abdel-Aziz M. and Abou El-Ella D.A., Design, synthesis, mechanistic studies and in silico ADME predictions of benzimidazole derivatives as novel antifungal agents, *Bioorg Chem*, **101**, 103956 **(2020)**

15. Mylonas S., Valavanidis A., Dimitropoulos K., Polissiou M., Tsiftsoglou A.S. and Vizirianakis I.S., Synthesis, molecular structure determination and antitumor activity of platinum(II) and palladium(II) complexes of 2-substituted benzimidazole, *J Inorg Biochem*, **34(4)**, 265 **(1988)**

16. Plumb D.C., Plumb's Veterinary Drug Handbook, Stockholm: John Wiley & Sons **(2018)**

17. Raghu M.S., Pradeep Kumar C.B., Yogesh Kumar K., Prashanth M.K., Alshahrani M.Y., Ahmad I. and Jain R., Design, synthesis and molecular docking studies of imidazole and benzimidazole linked ethionamide derivatives as inhibitors of InhA and antituberculosis agents, *Bioorg Med Chem Lett*, **60**, 128604 **(2022)**

18. Raied M. Shakir, Shaimaa A. Saoud, Husam S. Jasim and Dhuha F. Hussain, Synthesis, Antioxidant Activity And Molecular Docking Study of 1,2,4-Triazole And Their Corresponding Fused Rings Containing 2-Methylphenol, *IJDDT*, **11(2)**, 501 **(2021)**

19. Raied Mustafa Shakir, Khalid Fahad Ali and Dhuha Faruk Hussain, Synthesis and Antioxidant Ability of Some New 6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) Derivatives Bearing 2,6-Dimethoxy-4-(methoxymethyl)Phenol, *Oriental Journal Of Chemistry*, **33(5)**, 2492 **(2017)**

20. Rao V.K., Reddy S.S., Krishna B.S., Naidu K.R.M., Raju C.N. and Ghosh S.K., Synthesis of Schiff's bases in aqueous medium: a green alternative approach with effective mass yield and high reaction rates, *Green Chemistry Letters and Reviews*, **3(3)**, 217 **(2010)**

21. Ren M., Greenberg M.M. and Zhou C., Participation of Histones in DNA Damage and Repair within Nucleosome Core Particles: Mechanism and Applications, *Acc Chem Res*, **55(7)**, 1059 **(2022)**

22. Shaimaa Abed Saoud, Khalid Fahad Ali and Raied Mustafa Shakir, Relationship Between the Structure of Newly Synthesized Derivatives of 1,3,4-Oxadiazole Containing 2-Methylphenol and their Antioxidant and Antibacterial Activities, *Orient J Chem*, **33(4)**, 1781 **(2017)**

23. Shakir R.M., Synthesis and Antioxidant Ability of Some 4-(((4-(5-(Aryl)-1,3,4-oxadiazol-2-yl)benzyl)oxy)methyl)-2,6-dimethoxyphenol, *Oriental Journal Of Chemistry*, **32(5)**, 2611 **(2016)**

24. Shakir R.M., Ariffin A. and Ng S.W., Ethyl 4-[(3,5-di-tert-butyl-2-hydroxy-benzyl)amino]-benzoate, *Acta Crystallogr Sect E Struct Rep Online*, **66(Pt 11)**, o2916 **(2010)**

25. Tatipamula V.B. and Kukavica B., Phenolic compounds as antidiabetic, anti-inflammatory and anticancer agents and improvement of their bioavailability by liposomes, *Cell Biochem Funct*, **39(8)**, 926 **(2021)**

26. Tiglani D., Salahuddin, Mazumder A., Yar M.S., Kumar R. and Ahsan M.J., Benzimidazole-Quinoline Hybrid Scaffold as Promising Pharmacological Agents: A Review, *Polycyclic Aromat Compd*, **42(10)**, 4183 **(2022)**

27. Travascio P., Witting P.K., Mauk A.G. and Sen D., The Peroxidase Activity of a Hemin−DNA Oligonucleotide Complex: Free Radical Damage to Specific Guanine Bases of the DNA, *J Am Chem Soc*, **123(7)**, 1337 **(2001)**

28. Yildiz U., Antioxidant and DNA damage protecting activities of newly synthesized thiol bridged bis-benzimidazole derivative and its dicationic analogue, *J Heterocycl Chem*, **57(11),** 4007 **(2020)**

29. Zhang S., Yu Z., Xia J., Zhang X., Liu K., Sik A. and Jin M., Anti-Parkinson's disease activity of phenolic acids from Eucommia ulmoides Oliver leaf extracts and their autophagy activation mechanism, *Food and Function*, **11(2)**, 1425 **(2020)**.

(Received 28th May 2022, accepted 30th July 2022)