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Facile DES-mediated synthesis and antioxidant potency of benzimidazoquinazolinone motifs

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Abstract One-pot multicomponent synthesis of benzimidazoquinazolinone scaffolds was achieved by reaction between 2-aminobenzimidazole, aldehyde, and 1,3-cyclohexadione in choline chloride:glycerol as deep eutectic solvent (DES). The developed methodology offers mild and faster reaction conditions with excellent product yield. The synthesized compounds were screened for their antioxidant potency using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and metal chelating assay. Most of the compounds were found to exhibit good to comparable antioxidant activity with respect to standards. Use of this inexpensive and biodegradable solvent makes this methodology a greener approach compared with other methods reported in literature.

Graphical Abstract Greener, one-pot multicomponent synthesis of benzimidazoquinazolinone scaffolds using DES (ChCl:glycerol) as efficient, recyclable, and biodegradable solvent has been achieved. The synthesized compounds show good to comparable antioxidant activity compared with standards.

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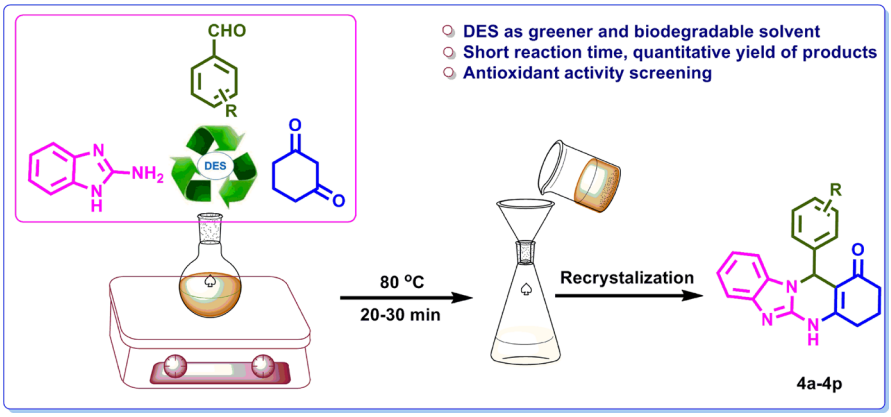


Keywords Benzimidazoquinazolinone · Deep eutectic solvent (DES) · Choline chloride:glycerol · Antioxidants

Introduction

In green chemistry, considerable attention is focused on development of clean, high-yielding, and environmentally friendly chemical processes and technologies [1, 2]. Over the last two decades, use of ionic liquids has attracted enormous attention from the scientific community for a variety of applications including catalysis, CO_2 absorption, etc. [3]. However, ionic liquids suffer from the disadvantages of high price, poor biodegradability, biocompatibility, toxicity, and environmental persistence [4, 5]. The cost and availability as well as tuning of anions always represent problems for synthetic research. From this point of view, alternative solvent systems are being investigated. In this respect, deep eutectic solvents (DESs) have been considered as alternatives to ionic liquids (ILs) [6, 7]. Generally, DESs are mixtures of a quaternary ammonium salt such as choline chloride with hydrogen-bond donors such as urea, carboxylic acid, and glycerol. DESs are environmentally benign, cheap, nontoxic, and biodegradable [8]. Their negligible vapor pressure, non-flammability, nonreactiveness with water, and synthesis from readily available raw materials makes them promising alternatives to ionic liquids (ILs) [9–11].

Over recent decades, multicomponent reactions (MCRs) have also gained significant importance in the field of medicinal chemistry as a tool for synthesis of a wide variety of biologically relevant scaffolds for generation of libraries of compounds [12–14]. Furthermore, MCRs using environmentally benign solvents such as DESs represent one of the most suitable strategies for green chemical processes and development of libraries of medicinal scaffolds [15–17]. Quinazolines and condensed quinazolines occupy a prominent position in the realm of medicinal chemistry because of their diverse medicinal properties, such as anticancer [18], histamine H1-receptor blocking [19], analgesic, antiinflammatory, antibacterial [20], antihypertensive [21], etc. actions. The general method for

synthesis of benzimidazoquinazolinone involves reaction of 2-aminobenzimidazole, aldehydes, and 1,3-cyclohexadione or dimedone. In literature, catalysts such as silica gel [22], *p*-TsOH·H_2O [23], I_2 [24], $H_6P_2W_{18}O_{62}$·$18H_2O$ [25], and NH_2SO_3H [26], and solvents such as dimethylformamide (DMF) [27, 28], ionic liquids such as [$bmim^+$][BF_4^-] [29] etc., have been reported for synthesis of benzimidazoquinazolinones. However, some of these methods are associated with limitations such as use of toxic solvent, drastic reaction conditions, long reaction time, and low product yield. Our group is constantly working on synthesis and bioactivity evaluation of benzimidazole compounds and development of greener synthetic approaches [30–32]. We report herein a catalyst-free approach for synthesis of benzimidazoquinazolinones using deep eutectic solvent (choline chloride:glycerol). Figure 1 shows important bioactive drugs containing benzimidazole, quinazolinone, and pyrimidine as core moieties in their structure. The synthetic strategies for synthesis of DES and the target derivatives (**4a**–**p**) are depicted in Schemes 1 and 2, respectively.

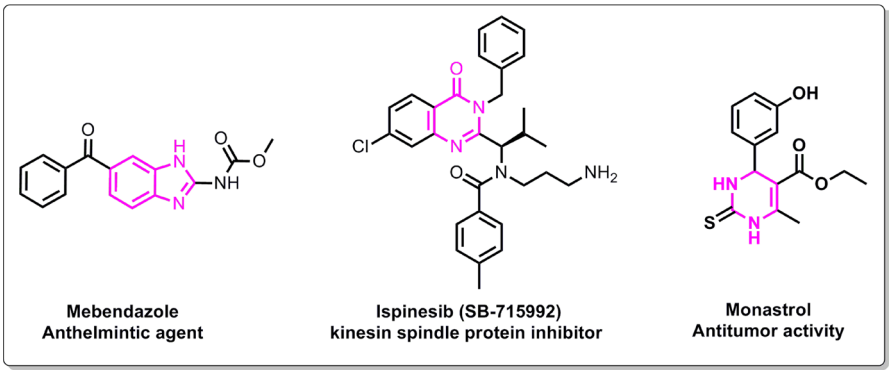
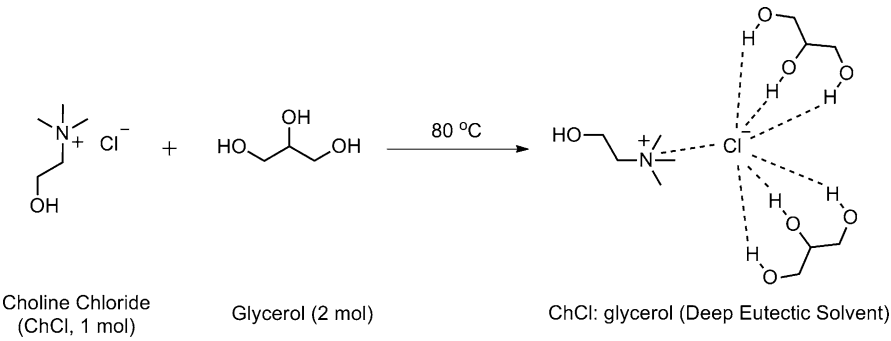
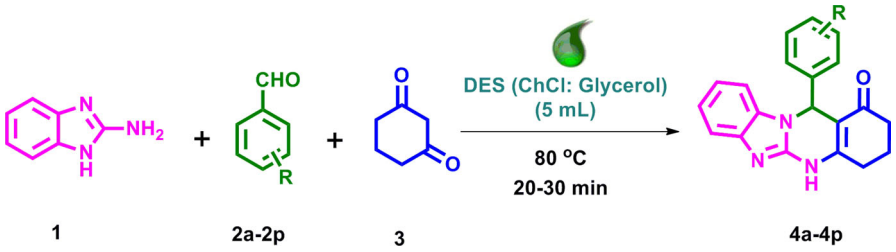


Fig. 1 Benzimidazole, quinazolinone and pyrimidine ring containing important bioactive molecules



Scheme 1 Preparation of deep eutectic solvent (ChCl:glycerol)



Scheme 2 General scheme for synthesis of benzimidazoquinazolinones in ChCl:glycerol

Results and discussion

Glycerol is a cheap, renewable, nonflammable, and biocompatible liquid, and choline chloride (quaternary ammonium salt) is a member of the vitamin B family, serving as a dietary supplement in animal feeds. Therefore, we decided to prepare DES (Scheme 1) from these two components (1:2 ratio) and study its applications as solvent for greener organic synthesis (Fig. 2).

Optimization of reaction conditions

The model reaction of 2-aminobenzimidazole, 3-nitrobenzaldehyde, and 1,3-cyclohexadione in ChCl:glycerol at 80 °C was carried out for synthesis of benzimidazoquinazolinone derivatives. The reaction was found to complete within 30 min to give the 3-nitrophenyl benzimidazoquinazolinone **4b** as product in quantitative yield (Table 2, entry 10). Encouraged by this result, we studied the effect of various solvents on the rate of the model reaction. Solvents such as ethanol, acetonitrile, DMF, chloroform, tetrahydrofuran (THF), glycerol, etc. were used for optimization of reaction conditions (Table 1). ChCl:glycerol was found to be an efficient solvent for synthesis of benzimidazoquinazolinone derivatives. However, reactions using other deep eutectic solvents such as ChCl:urea and ChCl:oxalic acid did not provide satisfactory results (Table 1). ChCl:glycerol has low viscosity and

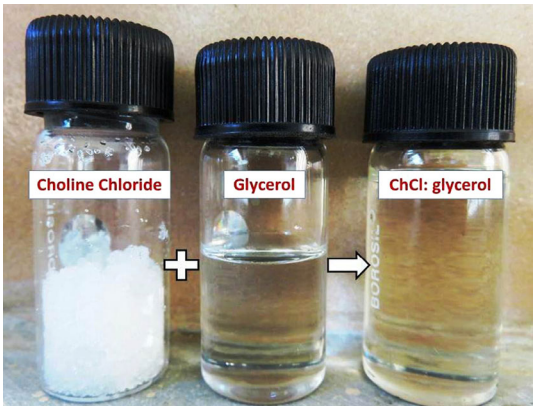


Fig. 2 Preparation of deep eutectic solvent (ChCl:glycerol)

Table 1 Reaction of 2-aminobenzimidazole **1**, 3-nitrobenzaldehyde **2b**, and 1,3 cyclohexadione **3** in different solvents

Entry	Solvent	Temp. (°C)	Time (min)	Yield of **4b** (%)[a]
1	Neat	80	180	31
2	Ethanol	Reflux	60	33
3	Acetonitrile	Reflux	180	56
4	DMF	120 °C	180	75
5	Chloroform	Reflux	240	70
6	THF	Reflux	240	57
7	Glycerol	90	180	77
8	ChCl:glycerol	RT	180	39
9	ChCl:glycerol	60	180	74
10[b]	ChCl:glycerol	80	30	91, 74, 63
11	ChCl:urea	80	120	41
12	ChCl:oxalic acid	80	120	42

Conditions: 2-aminobenzimidazole **1** (2 mmol), 3-nitrobenzaldehyde **2b** (2 mmol), 1,3-cyclohexadione (2 mmol), and DES (5 mL)

[a] Isolated yield

[b] DES was reused three times

low freezing point depression compared with ChCl:urea or ChCl:oxalic acid. Furthermore, the hydroxyl groups of glycerol impart more hydrogen bonding with choline chloride, hence it seems to promote reaction with higher product yield. The model reaction was performed at ambient temperature, but incomplete conversion of reactant into product was observed (Table 2, entry 8). When studied at elevated temperature of 80 °C, the model reaction afforded 91 % yield of **4b** within 30 min. To determine the efficiency and limitations of the DES in this multicomponent reaction, we applied the optimized reaction conditions (Table 2, entry 10) to a series of aldehydes. Aldehydes containing electron-donating as well electron-withdrawing groups such as hydroxyl, methoxy, and nitro could react efficiently to give corresponding products in good to quantitative yield (Table 2, entries 1–16). Furthermore, the efficiency of DES was evaluated by recycling it for subsequent reaction. Decrease in yield was observed after the second cycle. After reaction completion, the reaction mass was poured into crushed ice to obtain a solid product, which was filtered and dried. Water was removed from filtrate to obtain DES, which was then used directly as solvent in the next cycle. Thus, it was found that ChCl:glycerol plays a benign role of accelerator for reducing the reaction time and promoting effective product formation. The synthesized compounds were isolated, purified, and characterized by Fourier-transform infrared (FT-IR), ^{1}H nuclear magnetic resonance (NMR), and mass spectrometric techniques.

Study of Table 3 enables comparison of the efficiency of ChCl:glycerol (DES) versus reported methods. It is clear that the reported methodologies are associated with issues such as long reaction time, use of hazardous solvents, and reflux

Table 2 Synthesis of benzimidazoquinazolinones in deep eutectic solvent

Entry	R	Product	Time (min)	Yield (%)[a]	M.p. (°C)		References
					Found	Reported	
1	2-$NO_2C_6H_4$	**4a**	30	52	>300	>300	–
2	3-$NO_2C_6H_4$	**4b**	30	91	>300	>300	[33]
3	4-$NO_2C_6H_4$	**4c**	30	78	212	–	
4	4-$CH_3C_6H_4$	**4d**	30	84	>300	>300	[33]
5	4-$OCH_3C_6H_4$	**4e**	20	81	>300	>300	[33]
6	4-OH,3-$OCH_3C_6H_3$	**4f**	20	71	>300	–	
7	3-OH,4-$OCH_3C_6H_3$	**4g**	30	81	238–240	–	
8	1-Naphthyl	**4h**	30	85	>300	–	
9	4-BrC_6H_4	**4i**	30	78	>300	–	
10	2,3,4-$(OCH_3)_3C_6H_2$	**4j**	30	63	248–250	–	
11	4-$SCH_3C_6H_4$	**4k**	20	78	>300	–	
12	C_6H_5	**4l**	20	74	>300	>300	[33]
13	2,5-$(OCH_3)_2C_6H_3$	**4m**	30	89	260 (dec.)	–	
14	3,4-$(OCH_3)_2C_6H_3$	**4n**	30	75	>300	–	
15	4-OHC_6H_4	**4o**	30	85	>300	–	
16	3,4-$(OH)_2C_6H_3$	**4p**	45	60	262 (dec.)	–	

General reaction conditions: 2-aminobenzimidazole **1** (2 mmol), aldehydes **2a–p** (2 mmol), 1,3-cyclohexadione **3** (2 mmol), and ChCl:glycerol (5 mL). All products were characterized by IR, ^{1}H and ^{13}C NMR, and mass spectrometry

[a] Isolated yield

Table 3 Comparison of ChCl:glycerol with some reported methods

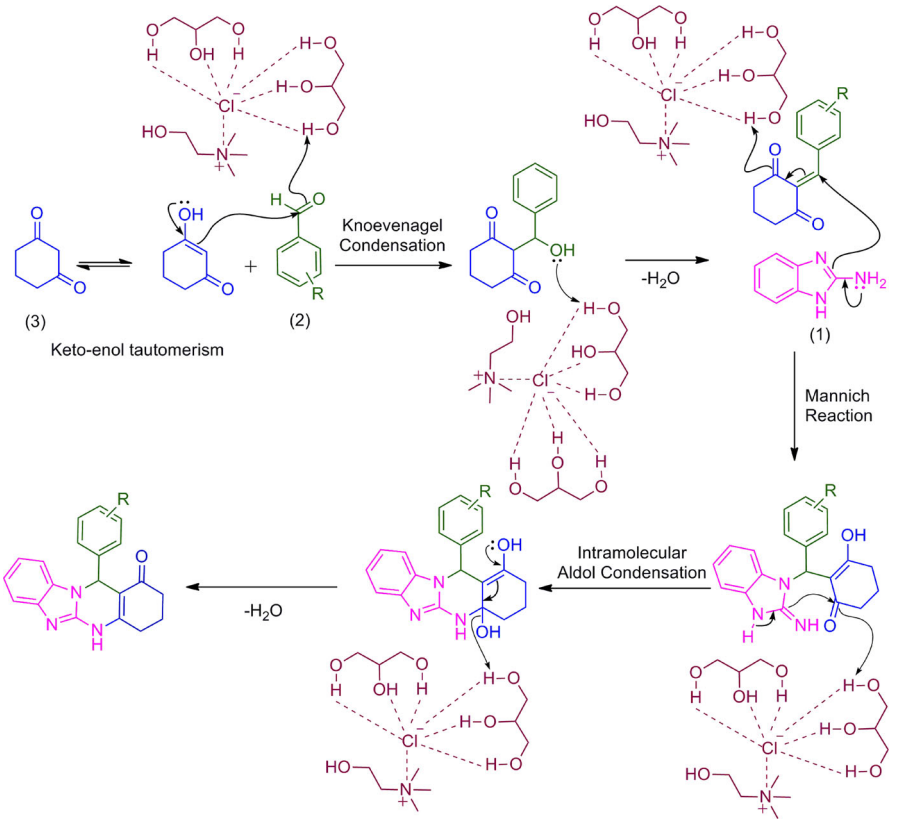
Entry	Catalyst	Condition	Time (min)	Solvent	Yield (%)
1	NH_2SO_3H	Reflux	20	CH_3CN	90 [26]
2	I_2	Reflux	10	CH_3CN	84 [24]
3	Ionic liquid	90 °C	360	–	84 [29]
4		Reflux	5	DMF	58 [27]
5	–	MW	5	DMF	89 [28]
6	–	Reflux	360	DMF	65 [28]
7[a, b]	–	80 °C	30	ChCl:glycerol	91 [This work]

[a] General reaction conditions: 2-aminobenzimidazole **1** (2 mmol), 3-nitrobenzaldehyde **2b** (2 mmol), 1,3-cyclohexadione **3** (2 mmol), and DES (5 mL)

[b] Isolated yield

conditions. These conditions are not preferable for green chemistry protocols. Hence, it is worth mentioning that the present work overcomes the drawbacks of reported methods.

We propose a mechanism for the synthesis of benzimidazoquinazolinones in Scheme 3. It is seen that DES plays an important role in promoting the reaction by



Scheme 3 Plausible mechanism for synthesis of benzimidazoquinazolinone derivatives

activation of carbonyl carbon of aldehyde (**2**) through hydrogen bonding and promoting Knoevenagel condensation between aldehyde (**2**) and 1,3-cyclohexadione (**3**) to form a Schiff base intermediate. In the next step, 2-aminobenzimidazole (**1**) reacts with the intermediate through Michael addition followed by cyclization and dehydration to give the desired product.

Antioxidant study

Measurement of antioxidant capacity is an easy task; however, determination of its mechanism is rather difficult. Antioxidants can act via many different mechanisms, such as hydrogen donation, electron donation, free-radical scavenging, and chelation of metal ions which can initiate free-radical reactions. Therefore, a single test for antioxidant capacity is not adequate [34].

DPPH radical scavenging activity

DPPH free-radical scavenging is a method regularly used to evaluate the antioxidant property of natural or synthetic compounds. This method is based on reaction of

DPPH with hydrogen donors to produce a stable product, causing a color change from purple to faint yellow [35]. The DPPH radical scavenging activity of the benzimidazoquinazolinone derivatives **4a–p** is depicted in Fig. 3. L-Ascorbic acid was used as positive control, and compounds **4a**, **b**, **d**, **f**, **g**, **i** showed excellent DPPH radical scavenging activity.

Ferric reducing antioxidant power (FRAP) assay

The benzimidazoquinazolinone derivatives **4a–p** were also evaluated using ferric reducing antioxidant power (FRAP). This reducing power assay monitors the electron-donating capacity of reducing agents (i.e., antioxidants) that cause reduction of the ferricyanide complex (Fe^{3+}) to ferrous (Fe^{2+}) ions, thereby generating chromogenic complex [36]. The spectral absorbance of the resultant blue–green-colored solution measured at 700 nm is proportional to the amount of Fe^{2+} in the mixture. The standard L-ascorbic acid showed 100 % reducing ability. Compounds **4a**, **f**, **g** showed moderate ferric reducing capacity, while **4p** exhibited excellent activity (Fig. 3).

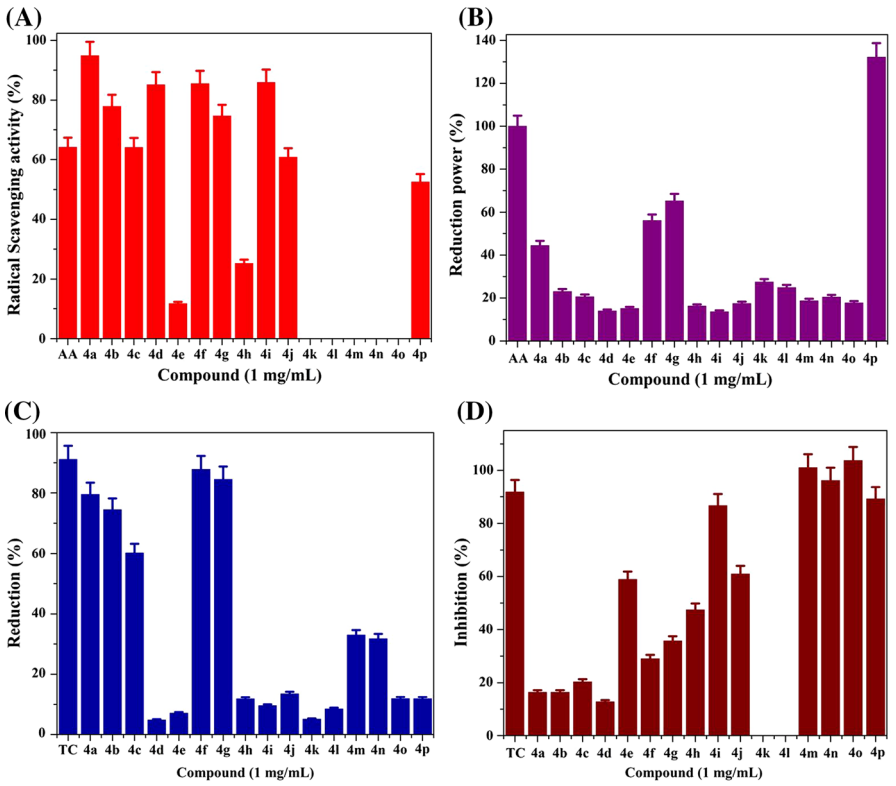


Fig. 3 In vitro antioxidant activity of benzimidazoquinazolinones, **a** DPPH radical scavenging assay, **b** ferric reducing antioxidant power (FRAP) assay, **c** ABTS, and **d** metal chelation assay (where *AA* ascorbic acid, *TC* α-tocopherol)

ABTS assay

In this study, the $ABTS^{\cdot+}$ radical cation scavenging capacity of the benzimidazoquinazolinone derivatives **4a–p** was evaluated. Stable radicals of ABTS are generated when ABTS is mixed with potassium persulfate and incubated in dark condition, as estimated spectrophotometrically at the characteristic wavelength of 734 nm for analysis of ABTS radicals [37]. Compounds **4f**, **g** showed comparable radical scavenging ability, while compounds **4a**, **b**, **c** were found to exhibit better activity. In all these analyses, α-tocopherol was used as standard.

Metal chelating ability

This assay studies complex formation between Fe^{2+} and ferrozine by measuring the decrease in violet color intensity when the metal chelating compound is present in solution. The decrease in the concentration of ferrous ions by benzimidazoquinazolinones provides protection against oxidative destruction of free radicals. The percentage iron chelating ability of α-tocopherol and the benzimidazoquinazolinones is shown in Fig. 3. Benzimidazoquinazolinone compounds **4i**, **m**, **n**, **o**, **p** showed significant metal chelating capacity compared with the standard α-tocopherol, while compounds **4e**, **j** exhibited moderate metal chelation activity.

All the radical scavenging activities are related to the ability of each antioxidant to neutralize oxidative stress created by oxidants. This can be achieved through various mechanisms such as hydrogen donation, electron donation, free-radical scavenging, and chelation of metal ions. In the present study, the antioxidant activity of the benzimidazoquinazolinones **4a–p** was studied by using DPPH, FRAP, ABTS, and metal chelating assays. From the obtained results it is noted that the compounds having hydroxyl, methoxy, and electron-withdrawing substituents in their scaffold showed good to comparable antioxidant activity with respect to standards. Figure 4 shows the correlation between the substituents of the benzimidazoquinazolinones and their antioxidant properties.

Experimental

Materials and methods

All solvents and reagents were purchased from commercial suppliers Sigma-Aldrich, S.D. Fine Chemicals Ltd., and Spectrochem India Ltd. and used without further purification. Reaction progress was monitored by thin-layer chromatography (TLC) on 0.2-mm precoated silica-gel 60 F254 plates (Merck, Germany), locating the spots using ultraviolet (UV) light as visualizing agent. Melting points were obtained by open capillary method and are uncorrected. IR spectra (KBr pellets) were recorded on a Shimadzu FT-IR-8400 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a FT-NMR Bruker Avance-II Spectrometer at 400 and 100 MHz, respectively, using dimethyl sulfoxide (DMSO)-d_6 as solvent. Mass spectra were recorded on Waters 2795 micromass Q-TOF micro and Waters 2995

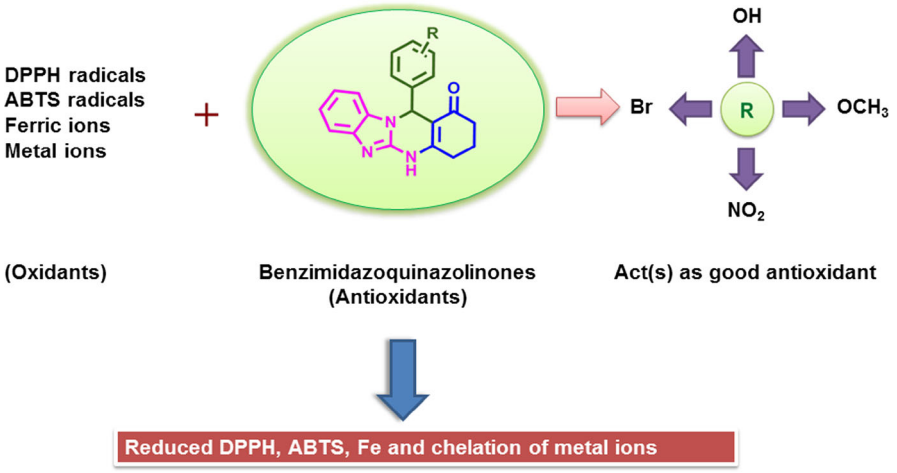


Fig. 4 Schematic representation of structure–activity correlation of benzimidazoquinazolinones

micromass quattro micro API mass spectrometer in electrospray ionization (ESI) mode. Antioxidant assays were performed using Shimadzu UV mini-1240 and Agilent Technologies Cary 60 UV–Vis spectrophotometers.

General procedure for preparation of deep eutectic solvent (DES)

Choline chloride (100 g, 0.71 mol) and glycerol (131.9 g, 1.43 mol) were placed in a 500-mL round-bottomed flask and stirred at 80 °C. After 15–20 min, a homogeneous colorless liquid solution was formed; this was cooled to room temperature and used in reactions without any purification (Scheme 1).

General procedure for synthesis of benzimidazoquinazolinone scaffolds

In a 50-mL round-bottomed flask, a mixture of 2-aminobenzimidazole **1** (2 mmol), aldehyde **2a**–**p** (2 mmol), 1,3-cyclohexadione **3** (2 mmol), and deep eutectic solvent (5 mL) was stirred at 80 °C for specified time (Scheme 2, Table 2). After reaction completion (monitored by TLC), the reaction mixture was cooled to room temperature then poured into crushed ice to obtain solid product, which was filtered, dried, and recrystallized from ethanol to afford pure product.

Spectral data of representative compounds

*12-(2-Nitrophenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4a**)*

Isolated as yellow powder; FT-IR (KBr) cm^{-1}: 3231, 3042, 2956, 2878, 2805, 1585, 1556, 1447, 1361, 1279, 1175, 1036, 806, 729; ^1H NMR (400 MHz, DMSO-d_6) δ_H: 1.98–1.79 (m, 2H, CH_2), 2.28–2.11 (m, 2H, CH_2), 2.68–2.65 (m, 2H, CH_2),

6.99–6.95 (m, 1H, ArH), 7.09 (s, 1H, CH), 7.12–7.07 (m, 2H, ArH), 7.33–7.31 (d, $J = 7.84$ Hz, 1H, ArH), 7.43–7.38 (m, 2H, ArH), 7.51–7.49 (m, 1H, ArH), 7.85–7.83 (d, $J = 8.12$ Hz, 1H, ArH), 11.32 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 196.03, 168.34, 142.16, 140.40, 133.78, 128.92, 124.06, 123.59, 122.96, 122.08, 117.34, 116.41, 114.41, 109.49, 96.73, 86.53, 57.84, 49.67, 30.07, 20.95; ESI MS $[M + H]^+$; *m/z* calcd. for $C_{20}H_{16}N_4O_3$: 361; found 361.

*12-(3-Nitrophenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4b**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3257, 3061, 2952, 2875, 1672, 1541, 1454, 1401, 1275, 1192, 1106, 877, 732; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.01–1.83 (m, 2H, CH_2), 2.37–2.19 (m, 2H, CH_2), 2.73–2.70 (m, 2H, CH_2), 6.66 (s, 1H, CH), 7.08–6.94 (m, 2H, ArH), 7.27–7.26 (d, $J = 7.88$ Hz, 1H, ArH), 7.41–7.39 (d, $J = 7.84$ Hz, 1H, ArH), 7.56–7.52 (m, 1H, ArH), 7.71–7.69 (d, $J = 7.88$ Hz, 1H, ArH), 8.05–8.02 (m, 1H, ArH), 8.26 (s, 1H, ArH), 11.33 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 193.04, 147.57, 144.96, 143.66, 133.42, 131.63, 130.05, 122.68, 122.08, 121.75, 120.74, 117.09, 109.99, 106.55, 53.38, 36.24, 26.54, 20.66; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{20}H_{16}N_4O_3$: 361; found 361, 379 $[M + H_2O]$.

*12-(p-Tolyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4d**)*

Isolated as light-yellow powder; FT-IR (KBr) cm^{-1}: 3222, 3027, 2879, 2804, 1584, 1440, 1361, 1262, 1195, 1012, 756; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.02–1.85 (m, 2H, CH_2), 2.19 (s, 3H, CH_3), 2.35–2.22 (m, 2H, CH_2), 2.74–2.62 (m, 2H, CH_2), 6.35 (s, 1H, CH), 6.95–6.91 (m, 1H, ArH), 7.05–7.01 (m, 3H, ArH), 7.20–7.17 (m, 3H, ArH), 7.36–7.34 (d, $J = 7.80$ Hz, 1H, ArH), 11.06 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 193.38, 138.69, 136.68, 131.29, 128.72, 127.48, 126.80, 124.67, 121.59, 120.33, 116.88, 113.80, 109.82, 107.82, 105.92, 87.25, 53.64, 28.99, 27.54, 20.66; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{21}H_{19}N_3O$: 330; found 330.

*12-(4-Methoxyphenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4e**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3217, 3032, 2890, 2814, 1584, 1445, 1361, 1256, 1032, 819, 742; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.02–1.84 (m, 2H, CH_2), 2.35–2.19 (m, 2H, CH_2), 2.73–2.62 (m, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.35 (s, 1H, CH), 6.77–6.75 (d, $J = 8.72$ Hz, 2H, ArH), 6.96–6.92 (m, 1H, ArH), 7.05–7.01 (m, 1H, ArH), 7.25–7.19 (m, 3H, ArH), 7.36–7.34 (d, $J = 7.80$ Hz, 1H, ArH), 11.05 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 192.75, 168.15, 167.39, 158.45, 145.37, 133.75, 131.96, 128.07, 121.58, 120.24, 113.48, 109.83, 107.68, 54.86, 53.41, 36.40, 26.49, 20.75; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{21}H_{19}N_3O_2$: 346; found 346.

*12-(4-Hydroxy-3-methoxyphenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4f**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3541, 3129, 3046, 2986, 2902, 2815, 1578, 1441, 1371, 1273, 1138, 1038, 757; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.01–1.87 (m, 2H, CH_2), 2.31–2.23 (m, 2H, CH_2), 2.71–2.66 (m, 2H, CH_2), 3.72 (s, 3H, OCH_3), 6.32 (s, 1H, CH), 6.59 (m, 2H, ArH), 7.06–6.94 (m, 3H, ArH), 7.30–7.28 (d, $J = 7.80$ Hz, 1H, ArH), 7.36–7.34 (d, $J = 7.84$ Hz, 1H, ArH), 8.87 (brs, 1H, OH), 11.02 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 192.84, 146.96, 145.93, 145.27, 141.96, 132.68, 131.96, 121.56, 120.21, 119.09, 116.77, 115.21, 111.74, 110.01, 107.75, 55.61, 53.66, 36.45, 26.52, 20.77; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{21}H_{19}N_3O_3$: 362; found 362.

*12-(3-Hydroxy-4-methoxyphenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4g**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3485, 3305, 3226, 2963, 2886, 1719, 1625, 1585, 1443, 1359, 1273, 1033, 761; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.02–1.82 (m, 2H, CH_2), 2.35–2.14 (m, 2H, CH_2), 2.74–2.61 (m, 2H, CH_2), 3.67 (s, 3H, OCH_3), 6.28 (s, 1H, CH), 6.68 (s, 1H, ArH), 6.75–6.71 (m, 2H, ArH), 6.97–6.93 (m, 1H, ArH), 7.06–7.02 (m, 1H, ArH), 7.20–7.18 (d, $J = 7.76$ Hz, 1H, ArH), 7.37–7.35 (d, $J = 7.84$ Hz, 1H, ArH), 8.79 (brs, 1H, OH), 11.06 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 192.73, 151.66, 146.97, 146.19, 145.33, 141.95, 134.19, 131.92, 121.57, 120.22, 117.92, 116.79, 111.93, 111.47, 109.83, 107.75, 55.40, 53.47, 36.43, 26.53, 20.73; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{21}H_{19}N_3O_3$: 362; found 362.

*12-(Naphthalen-1-yl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4h**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3227, 3039, 2956, 2889, 2808, 1625, 1586, 1443, 1361, 1258, 1175, 763; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.01–1.84 (m, 2H, CH), 2.35–2.11 (m, 2H, CH), 2.79–2.70 (m, 2H, CH), 6.81–6.73 (m, 2H, ArH), 6.95 (s, 1H, CH), 6.97–6.93 (m, 1H, ArH), 7.22 (brs, 1H, ArH), 7.34–7.32 (d, $J = 7.96$ Hz, 1H, ArH), 7.41–7.40 (m, 1H, ArH), 7.55–7.51 (m, 2H, ArH), 7.65 (brs, 1H, ArH), 7.79–7.76 (d, $J = 8.44$ Hz, 1H, ArH), 7.91–7.89 (d, $J = 7.96$ Hz, 1H, ArH), 11.27 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: because of less solubility of compound, ^{13}C NMR could not be obtained; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{24}H_{19}N_3O$: 366.43; found 366.26.

*12-(2,3,4-Trimethoxyphenyl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-one (**4j**)*

Isolated as white powder; FT-IR (KBr) cm^{-1}: 3221, 2947, 2827, 1584, 1442, 1359, 1273, 1102, 1030, 895, 750; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.06–1.81 (m, 2H, CH), 2.37–2.15 (m, 2H, CH), 2.75–2.63 (m, 2H, CH), 3.67 (s, 3H, OCH_3), 3.71

(s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.47 (s, 1H, CH), 6.70–6.68 (d, $J = 8.76$ Hz, 1H, ArH), 7.05–6.92 (m, 3H, ArH), 7.28–7.26 (d, $J = 7.84$ Hz, 1H, ArH), 7.34–7.32 (d, $J = 7.76$ Hz, 1H, ArH), 11.05 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: because of less solubility of compound, ^{13}C NMR could not be obtained; ESI–MS $[M + H]^+$; *m/z* calcd. for $C_{23}H_{23}N_3O_4$: 406.45; found 406.29.

Experimental protocols for antioxidant activity

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method described by Wu et al. [38] was used to assay the free radical scavenging ability of benzimidazoquinazolinone derivatives **4a–p** with modifications. One milliliter of 0.2 mM DPPH reagent prepared in methanol was added in tubes containing 0.8 mL of each compound (1 mg mL^{-1} in DMSO), and the mixture was allowed to stand for 30 min in the dark at room temperature. Similarly, the same protocol was performed for L-ascorbic acid as standard antioxidant. The absorbance of the resulting mixture was measured at 517 nm by UV–Vis spectrophotometer. Control was prepared by adding only DMSO to DPPH reagent, and the analysis was performed as described above. The % scavenging activity was determined as follows:

$$\text{Radical scavenging activity } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorption of control (blank, only DMSO) and A_1 is the absorption of compound **4a–p**.

Ferric reducing antioxidant power (FRAP) assay

The FRAP ability of the compounds was determined using the protocol described by Udayaprakash et al. [39] with modifications. One milliliter of compound (1 mg mL^{-1}), 2.5 mL phosphate buffer (0.1 M, pH 7), and 1 % potassium ferricyanide (2.5 mL) were mixed together and incubated at 50 ± 2 °C for 30 min. To the solution, 2.5 mL of 10 % trichloroacetic acid was added and centrifuged at 7000 rpm for 10 min. Distilled water (2.5 mL) and 0.5 mL of 0.1 % $FeCl_3$ were added to 2.5 mL supernatant. The absorbance of the solution was measured at 700 nm using UV–Vis spectrophotometer. In all experiments, L-ascorbic acid was used as standard. The percentage reduction of the compound as compared with standard was calculated using the following equation:

$$\text{Percentage of reduction power} = [1 - (1 - A_s/A_c)] \times 100$$

where A_c is the spectral absorption of the standard at the maximum concentration tested and A_s is the absorption measured for the compound.

ABTS activity

The ABTS ability of the compounds was determined using the protocol described by Udayaprakash et al. [39] with modifications. Solution of 7 mM 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and potassium persulfate (2.45 mM) was prepared and incubated in the dark for 14 h, after which the solution was diluted with absolute ethanol until the optical absorbance at 734 nm reached 0.7 ± 0.001. In a fresh test tube, one milliliter of diluted solution was mixed with 100 μL compound (1 mg mL^{-1}), and after 5 min the absorbance was measured at 734 nm. α-Tocopherol was used as standard. The percentage reduction ($I\,\%$) against ABTS was calculated using the following equation:

$$\text{Percentage reduction } (I\,\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the optical absorption of control (blank, only DMSO) and A_1 is the absorption obtained for the compound.

Metal chelating activity

The metal chelating ability of the synthesized compounds **4a**–**p** was determined using the protocol described by Nagulendran et al. [40] with modifications. Ferrous chloride (50 μL of 2 mM) was added into test tubes containing 400 μL of compound (1 mg mL^{-1}). The reaction was started by adding ferrozine (200 μL of 5 mM), and the final volume of the mixture was adjusted to 4.0 mL using absolute ethanol. The mixture was shaken and incubated at RT for 10 min. To determine the ferrous chelating activity of compounds, the spectral absorbance of the mixture was measured by UV–Vis spectrophotometer at 562 nm. α-Tocopherol was used as standard. The percentage inhibition of ferrozine–Fe^{2+} complex formation ($I\,\%$) was calculated using the following equation:

$$\text{Radical scavenging activity } (I\,\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorption of control (blank, only DMSO) and A_1 is the absorption of the compound.

Conclusions

We successfully developed a catalyst-free protocol for environmentally benign synthesis of benzimidazoquinazolinones using ChCl:glycerol as solvent. The novelty of this work is the use of a nontoxic, low-cost, and environmentally benign solvent. The methodology offers benefits such as clean and mild reaction conditions, short reaction time, and quantitative product yield. Furthermore, it is noteworthy that most of the synthesized compounds exhibited acceptable antioxidant activity.

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