Scientific paper

Microwave-assisted One-pot Efficient Synthesis of Functionalized 2-Oxo-2-phenylethylidenes-linked 2-Oxobenzo[1,4]oxazines and 2-Oxoquino[1,4]oxalines: Synthetic Applications, Antioxidant Activity, SAR and Cytotoxic Studies

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Abstract

A microwave-assisted, environmentally benign green protocol for the synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3, 4-dihydro-2H-benzo[b][1,4]oxazin-2-ones (**11a–n**) in excellent yields (upto 97%) and (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-ones (**14a–h**) (upto 96% yield) are reported. The practical applicability of developed methodology were also confirmed by the gram scale synthesis of **11a**, **14c** and **14e**; synthesis of anticancer alkaloid Cephalandole A **16** (89% yield). All the synthesized compounds **11a–n**, **14a–h** and **16** were assessed for their *in vitro* antioxidant activities in DPPH radical scavenging and FRAP assay. In DPPH assay, compounds **11a**, **14c** and **14e**, the most active compounds of the series, were found to show IC₅₀ value of 10.20 ± 0.08 µg/mL, 9.89 ± 0.15 µg/mL and 8.97 ± 0.13 µg/mL, respectively in comparison with standard reference (ascorbic acid, IC₅₀ = 4.57 µg/mL). Whereas, in FRAP antioxidant assay seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**) displayed higher antioxidant activity in comparison to the reference standard BHT (C_{0.5}FRAP = 546.2 µM). Moreover, the cytotoxic studies of the compounds **11a**, **14c**, **14e** and **14h** were found to be non-toxic in nature in 3T₃ fibroblast cell lines using MTT assay.

Keywords: Benzo[1,4]oxazines; 2-oxobenzo[1,4]oxazines; 2-oxoquino[1,4]oxalines; Antioxidant; Microwave-Assisted Organic Synthesis (MAOS); DPPH; FRAP; Structure-Activity Relationship.

1. Introduction

Benzo[1,4]oxazines **1–8**, a sub-class of benzo fused heterocycles, are endowed with a wide range of biological activities such as anti-inflammatory,¹ analgesic,² antibacterial,³ neuroprotective,⁴ D2 receptor antagonists,⁵ antimycobacterial,^{6,7} antihypertensive,⁸ antifungal,⁹ herbicidal,¹⁰ antiarrhythmic,¹¹ thrombin inhibitor and fibrinogen receptor antagonists,¹² 5-HT receptor antagonists,¹³ potent inhibitor of tumor-driven angiogenesis^{14a} and selective non-steroidal mineralocorticoid receptor

antagonists^{14b} etc. Some marine secondary metabolites such as, Arcticoside **5a** (potent antifungal agent) and C-1027 chromophore- III & V **5b** (potent antitumor antibiotic),¹⁵ which were isolated from a culture of an arctic marine actinomycete *Streptomyces* strain; possess as benzo[1,4]oxazines substructures in their active scaffolds (figure 1).

Owing to the several biological activities having benzo[1,4]oxazines moieties in their scaffold or in whole molecule, several syntheses of benzo[1,4]oxazines, 2-oxo-

Figure 1. Structures of biologically active benzo[1,4]oxazines **1–8**.

benzo[1,4] oxazines and its related structural motifs have been reported in the literature using metal as well as metal-free catalyst.^{16–18} Earlier, Kikelj et al. reported the first synthesis of 3-unsubstituted 3,4-dihydro-1,4-benzoxazin-2-ones *via* catalytic hydrogenation of 4-benzyl-3,4-dihydro-1,4-benzoxazin-2-one.^{16a} Since then, several metal-catalysed synthesis of substituted benzo[1,4]oxazines have been reported in the literature^{16b–d} [figure 2; entry i–iii]. 2-aminophenols or substituted 2-nitrophenols^{16f} or 2-halophenols,^{16g–j} were most commonly used as starting materials towards the synthesis of benzo[1,4]oxazine derivatives. With 2-aminophenols as the starting substrate, various protection and deprotection steps are required.^{16k–l} Xia et al. (2008) reported sulphamic acid as an efficient catalyst for the synthesis of benzo[1,4]oxazines derivatives in one pot reaction condition providing good yield.^{16e} In spite of this efficient methodology, sulphamic acid is associated with several drawbacks with respect to its hazardous nature towards animals as well as environment, such as the high toxicity of sulphamic acid in animals (LD₅₀ = 1312 mg/kg in mouse via oral route; LD₅₀ = 3160 mg/kg in rat via oral route; toxicity value of LC₅₀ = 70.3 mg/l in fish *Pimephales promelas* species); acute oral and inhalation toxicity to human etc. Its disposal also induces toxicity to the environment.^{16f} Thus, none of the reported methodologies were environmentally benign as these were associated with several drawbacks such as the

use of toxic catalysts, toxic starting materials, hazardous organic solvents, multistep and complicated reaction assembly, limited number of appropriate substrates for diverse synthesis, tedious workup and low yields etc.^{19–21} Therefore, an efficient, environmentally benign and more green approach for the synthesis of benzo[1,4]oxazines is still a challenging area of research.

Moreover, it has also been observed that several chalcones and its analogues,^{22a–c} quinolines,^{22d–f} and coumarin-derived scaffolds,^{22g,h} which have 2-oxobenzo[1,4]oxazine-like substructure in their scaffold, were found to be potent antioxidants under several *in vitro* antioxidant assays. So, in our endeavour to search for new class of potent antioxidants we have developed inclinations towards 2-oxo-benzo[1,4]oxazine based analogues (prototype A: figure 1), because it has almost similar substructure as present in coumarins, chalcones and quinolone or its analogues. In this context, we were interested to explore the green synthesis and antioxidant activity of non-naturally occurring 2-oxobenzo[1,4]oxazines derived analogues, because to the best of our knowledge the antioxidant activity of 2-oxobenzo[1,4]oxazine class of molecules have never been studied earlier.

During the past few decades, Microwave-Assisted Organic Synthesis (MAOS) has been identified as an efficient green protocol for accelerating drug discovery process.^{23a–d} Moreover, it is well documented that Mi-

Figure 2. Previous and present reports for the synthesis of substituted benzo[1,4]oxazine derivatives.

crowave irradiation (MW) is a form of electromagnetic energy having lower frequency (300–300000 MHz) and it has several advantages over conventional heating conditions such as: reduction of the reaction times as well as decrease side reactions, increase yields of desired products and improve reproducibility. Therefore, industrial as well as academic research groups are frequently using MAOS for rapid reaction optimization, accelerating the efficient synthesis of new chemical entities and also for the novel methodology development.^{23e–g} Hence, utilizing this concept; herein, we report a very simple, mild and highly efficient green protocol for the synthesis of highly functionalized 2-oxobenzo[1,4]oxazines **11a–n** (upto 97% yield) and 2-oxoquino[1,4]oxalines **14a–h** (upto 96% yield) under microwave irradiations using readily available starting materials. The main advantage of this protocol is the avoidance of any toxic reagent, solvent or catalyst. Although, compounds **11a–i** and **14a–h** are already reported in the literature, but they were prepared by other routes,¹⁷ and their antioxidant activities have never been evaluated till now. Therefore, for the first time, we have evaluated the *in vitro* antioxidant activities of all the synthesized compounds **11a–n**, **14a–h** and **16** in DPPH radical scavenging assay using ascorbic acid ($IC_{50} = 4.57 \mu\text{g/mL}$) as standard reference and ferric reducing antioxidant power (FRAP) assay taking BHT ($546.0 \pm 13.6 \mu\text{M}$) as standard reference.

To the best of our knowledge, this is the first report of microwave-assisted synthesis and *in vitro* antioxidant activities of functionalized 2-oxobenzo[1,4]oxazines **11a–n**, 2-oxoquino[1,4]oxalines **14a–h** and **16** in excellent yields having high level of functional group compatibility.

2. Results and Discussion

We started our initial investigation towards the development of an environmentally benign, sustainable protocol for the synthesis of 2-oxobenzo[1,4]oxazine with a typical model reaction between 2,4-dioxo-4-phenylbutanoic acid **9a** and 2-aminophenol **10a** in isopropanol (1.0 mL) under nitrogen atmosphere at room temperature for 3 h which furnished the condensation product **11a** in only 18% yield (entry 1, Table 1). Carrying out the above reaction at 90 °C for 3 h afforded **11a** in better yield [(45%), entry 2, Table 1, Method 1]. The product obtained was fully characterized by its spectroscopic data (¹H and ¹³C NMR, HRMS and IR).

Since we observed an increase in the yield of **11a** as we change solvent from isopropanol to DMF and conventional to MW irradiation condition; we switched over to more polar DMSO solvent. Thus, the above reaction was carried out in DMSO solvent, at 150 °C for 10 min under microwave irradiation, which furnished **11a** in 77% yield (entry 7, Table 1, Method B). Decreasing or increasing the

Table 1. Optimization study: Synthesis of 2-oxobenzo[1,4]oxazines **11a** by the reaction of 2,4-dioxo-4-phenylbutanoic acid **9a** and 2-aminophenol **10a**.^a

Entry	Solvent	Temp (°C)	Method A ^b		Method B ^c	
			Time (min)	Yield (%) ^d	Time (min)	Yield (%) ^d
1	Isopropanol	rt	180	18	–	–
2	Isopropanol	90	180	45	10	52
3	Isopropanol	90	300	55	30	58
4	DMF	90	180	51	30	64
5	DMF	120	180	58	20	62
6	DMF	150	120	50	15	69
7	DMSO	150	180	59	10	77
8	DMSO	150	240	61	5	51
9	DMSO	150	300	67	15	72
10	DMSO	180	120	65	2	56
11	Diethylene glycol	150	180	61	5	94
12	Diethylene glycol	150	120	54	3	80
13	Diethylene glycol	150	300	67	7	93
14	Diethylene glycol	170	180	64	2	85
15	Diethylene glycol	160	180	65	2	82
16	Diethylene glycol	170	300	63	5	81

^a Reaction conditions: **9a** (0.1 mmol), **10a** (0.1 mmol) in solvent (1.0 mL), 5–300 min, N₂ atmosphere. ^b Method A: Conventional heating;

^c Method B: Microwave Irradiation; ^d Isolated yield after recrystallization/column chromatography.

^a unless otherwise mentioned, all the reactions were carried out with substrates **9a-f** (0.2 mmol), substituted 2-aminophenols **10a-c** (0.2 mmol) in diethylene glycol (2.0 mL) at 150 °C under microwave irradiation. ^b Isolated yield.

Scheme 1. Microwave-assisted one-pot green synthesis of 2-oxobenzo[1,4]oxazine analogues (**11a-n**).^{a,b}

time for the above reaction in DMSO solvent was not found to be fruitful. (Entries 8–10, Table 1). Then, we planned to perform our model reaction in more polar diethylene glycol as solvent. To our surprise, after 5 min at 150 °C, we obtained **11a** in 94% yield (entry 11, Table 1). Furthermore, in spite of increasing the reaction temperature from 150 °C to 170 °C or increasing/ decreasing the reaction time; we were successful in obtaining **11a** in the yield range of 81–93%

(entries 12–16, Table 1). Finally, based on above screening studies, diethylene glycol as solvent, 150 °C temperature for 5 min was found to be the best optimized reaction condition under microwave irradiations (entry 11, Table 1).

After optimization study, we further investigated the scope and generality of this reaction. Several alkyl/alkoxy/halide/nitro-substituted 2,4-dioxo-4-phenylbutanoic acids **9a–f** were reacted with alkyl/halide/nitro-substituted

^a Unless otherwise mentioned, all reactions were carried out with substrates **9a–g** or **12** (0.2 mmol) and 1,2-diamino benzene **13** (0.2 mmol) in diethylene glycol (2.0 mL) at 150 °C under microwave irradiation. ^b Isolated yield.

Scheme 2. Microwave-assisted one-pot synthesis of functionalized 2-oxoquino[1,4]oxalines **14a–h**.^a

ed 2-aminophenol **10a–c** in diethylene glycol under our optimized conditions (Scheme 1). The desired 2-oxobenzo[1,4]oxazines **11a–n** were purified either by flash column chromatography method or by recrystallization (see experimental section).

As evident from scheme 1; substituted 2, 4-di-oxo-4-phenylbutanoic acid **9a–f** reacted smoothly with substituted 2-aminophenol **10a–c**, and furnished substituted 2-oxobenzo[1,4]oxazines **11a–n** in 78–97% yield range. It has been observed that nitro-based 2-oxobenzo[1,4]oxazines **11k–n** were obtained in comparatively lesser yields (78–83%) with rest of the compounds **11a–j**. This is due to poor solubility of nitro-based 2-oxobenzo[1,4]oxazines **11k–n** in ethyl acetate which makes the purification of these compounds via column chromatography very tedious and cumbersome. In this study, the most characteristic feature observed was that a broad range of functional groups, like Cl, Br, OMe and NO₂ are well compatible under our optimized reaction conditions. Thus, these groups can further be manipulated to obtain new therapeutic molecules.

After successful implementation of our methodology on **11a–n** series; we extended its synthetic application towards the synthesis of its congener class of bioactive heterocycles i.e. 2-oxoquino[1,4]oxalines **14a–h**; which were synthesized from **9a–g** with phenyl-1,2-diamine **13** using our optimised methodology in the excellent yield (90–96%), as depicted in Scheme 2.

Furthermore, the practicality of this methodology was demonstrated via gram scale synthesis of compounds **11a**, **14c** and **14e**. Thus, the reaction of **9a** (2.00 g, 10.40 mmol), **9c** (2.26 g, 10.00 mmol) or **9e** (2.71 g, 10.00 mmol) with either **10a** (1.13 g, 10.40 mmol) or **13** (1.08 g,

10.00 mmol) in diethylene glycol under MW irradiation at 150 °C for 7–9 min furnished the target compounds, **11a** (2.49 g, 90.41%); **14c** (2.60 g, 87.32%) and **14e** (3.09 g, 90.14%), respectively. (Scheme 3)

We have further demonstrated practicality of our developed methodology for the synthesis of anticancer indole alkaloid, Cephalandole A, which was isolated from Taiwanese orchid *Cephalanceropsis gracilis* (Orchidaceae). Its crude extract showed good activity against CNS (SF-268; IC₅₀ = 12.2 µM), breast (MCF-7; IC₅₀ = 7.57 µM) and lung (NCI-H460; IC₅₀ = 7.8 µM) carcinoma cell lines.²⁴ 3-indoleglyoxylic acid (**15**) on reaction with aminophenol (**10a**) in diethylene glycol under MW at 150 °C for 10 min furnished indole alkaloid Cephalandole A (**16**) in 89% yield (Scheme 4). The spectral data was found to be the same as the literature data.

2. 1. Material and Methods for Antioxidant Activity

2. 1. 1. *In vitro* antioxidant DPPH radical scavenging activity^{24a–e}

In DPPH radical scavenging method the synthesized compounds at different concentrations ranging from 10 to 100 µg mL⁻¹ was mixed with 1.5 mL of a DPPH methanolic solution (20 mg L⁻¹). Pure methanol was taken as control and ascorbic acid (vitamin C) was used as a reference compound. The percent of DPPH decoloration of the sample was calculated according to the formula.

$$\text{Decoloration \%} = [1 - (\text{Abs sample} / \text{Abs control})] \times 100$$

Scheme 3. Gram scale synthesis of **11a**, **14c** and **14e**.

Scheme 4. Synthesis of Cephalandole A (**16**)

The decoloration was plotted against the sample concentration and a logarithmic regression curve was established in order to calculate the IC_{50} . The results are expressed as antiradical efficiency (AE), which is 1000-fold inverse of the IC_{50} value $AE = 1000 / IC_{50}$.

2. 1. 2. *In vitro* Ferric Reducing Antioxidant Power (FRAP) Assay^{24f}

The FRAP reagent was prepared by mixing freshly prepared 10.0 mM of ferric-tripyridyltriazine (TPTZ) solution, 20.0 mM $FeCl_3 \times 6H_2O$ solution and 300 mM sodium acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). Sample was added to 3 mL of FRAP reagent and this reaction mixture was incubated for 30 min at 37 °C temperature. The absorbance of prepared reaction mixture was measured at 593 nm. A freshly prepared solution of $FeSO_4$ was used for calibration of standard curve. The FRAP antioxidant capacities were expressed in terms of $C_{0.5FRAP}$ (the concentration of samples with respect to the antioxidant ability equivalent to that of $FeSO_4$ at 0.5 mmol/L).

2. 2. Antioxidant Activity: *In vitro* antioxidant DPPH Radical Scavenging Assay, FRAP Assay and Structure-activity Relationship²⁵

The DPPH radical scavenging assay is generally utilised for the screening of antioxidant activity of diverse heterocycles.^{25a} DPPH is a stable free radical, which can easily accept an electron or a hydrogen radical to become a stable molecule. Literature reports illustrate that DPPH assay works in two ways; a single electron transfer (SET) or a hydrogen atom transfer (HAT) mechanism.^{25b} DPPH in the methanolic medium has odd electron configuration which shows a strong absorption band at 515 nm and the absorbance decreases in the presence of free radical scavengers which results in the colour change from deep purple to yellow.^{25c-d} The radical quenching ability strongly depends on the structural accessibility of the radical trapping site. The electron density as well as steric hindrance plays a vital role in the antioxidant activity because they may prevent the test molecule from reaching to the DPPH radical site and thus results in lower activity.^{25e}

Table 2. Antioxidant activity of synthesized compounds **11a–n**, **14a–h** and **16** by DPPH radical scavenging and FRAP assay.²⁵

S. No.	Compound No.	Antioxidant activity ^a	
		DPPH assay (IC_{50}) (μ g/mL)	FRAP assay ($C_{0.5FRAP}$) (μ M)
1	11a	10.20 $\pm$ 0.08	611.5 $\pm$ 23.2
2	11b	19.70 $\pm$ 0.31	763.2 $\pm$ 38.1
3	11c	29.80 $\pm$ 0.17	306.8 $\pm$ 25.8
4	11d	65.32 $\pm$ 0.97	>1000
5	11e	25.02 $\pm$ 0.21	421.7 $\pm$ 37.9
6	11f	23.45 $\pm$ 0.14	845.9 $\pm$ 35.1
7	11g	67.40 $\pm$ 0.28	>1000
8	11h	78.50 $\pm$ 1.41	921.6 $\pm$ 29.6
9	11i	34.42 $\pm$ 0.62	291.7 $\pm$ 23.1
10	11j	42.98 $\pm$ 0.76	>1000
11	11k	21.27 $\pm$ 0.11	489.2 $\pm$ 18.5
12	11l	56.12 $\pm$ 1.03	348.8 $\pm$ 31.4
13	11m	15.70 $\pm$ 0.14	598.5 $\pm$ 23.4
14	11n	78.76 $\pm$ 1.43	>1000
15	14a	27.36 $\pm$ 0.44	638.4 $\pm$ 37.6
16	14b	91.36 $\pm$ 2.04	>1000
17	14c	9.89 $\pm$ 0.15	612.8 $\pm$ 17.8
18	14d	28.24 $\pm$ 0.46	498.4 $\pm$ 22.4
19	14e	8.97 $\pm$ 0.13	689.3 $\pm$ 30.0
20	14f	43.54 $\pm$ 0.88	592.7 $\pm$ 41.6
21	14g	38.97 $\pm$ 0.97	>1000
22	14h	14.27 $\pm$ 0.23	358.3 $\pm$ 17.7
23	16	11.87 $\pm$ 0.14	ND ^b
24	Ascorbic acid	4.57	–
25	BHT	–	546.0 $\pm$ 13.6

^aResults are expressed as a mean $\pm$ standard deviation ($n = 3$). DPPH radical scavenging activities are expressed as IC_{50} concentrations of the compounds (μ g/mL) required to inhibit 50 % of the radicals and the maximum inhibition values;

^bND means not done.

The FRAP assay was measured using the method as described by Benzie and Strain.^{25f} This methodology demonstrates that the antioxidant molecule reacts to a complex of ferric tripyridyltriazine [Fe^{3+} -TPTZ] and produces a colored ferrous tripyridyltriazine [Fe^{2+} -TPTZ] complex. Generally, the reducing nature of antioxidant molecule is associated with their action by breaking the free radical chain via donating a hydrogen atom.

All the synthesized 2-oxobenzo[1,4]oxazines **11a–n**, 2-oxoquino[1,4]oxalines **14a–h** and Cephalandole A **16** were screened for their *in vitro* antioxidant activities using DPPH radical scavenging activity assay using ascorbic acid as standard reference as well as in FRAP assay using BHT as standard reference (Table 2).^{25,26}

The *in vitro* antioxidant screening of compounds **11a–f**, having no substitution at benzoxazine aromatic ring; it had been observed that the compound **11a** was found to be the most active compound having IC_{50} value of 10.20 ± 0.08 $\mu\text{g/mL}$ (entry 1, Table 2) in comparison with standard reference ascorbic acid ($\text{IC}_{50} = 4.57$ $\mu\text{g/mL}$). When mono-halo substituents were present at side chain of aromatic ring as in case of **11c**, **11e** and **11f** having $-\text{Cl}$, $-\text{F}$ and $-\text{Br}$ substituent respectively; these molecules exhibited slightly lesser antioxidant activity in comparison with **11a** (Table 2; entry 3, 5 and 6). Further, 2-oxobenzo[1,4]oxazine having di-halo substituents on side chain of aromatic ring (**11d**) exhibited decrease of antioxidant activity drastically (Table 2; entry 4) in comparison to mono-halo substituted analogues **11c**, **11e** and **11f**. In addition, electron-donating substituents at side chain of aromatic ring in compound **11b** showed better antioxidant activity in comparison to halo-substituted 2-oxobenzo[1,4]oxazine analogues **11c–11f**.

Moreover, in 2-oxobenzo[1,4]oxazines having $-\text{CH}_3$ or $-\text{NO}_2$ substituent at para-position of benzoxazine aromatic nucleus (**11g–11n**); the antioxidant activity was found to be lesser (Table 2; entry 7–14) in comparison with unsubstituted analogues **11a–11f**, except compound **11k** and **11m**; which exhibited better activity profile (Table 2; entry 11 and 13). Whereas, 2-oxobenzo[1,4]oxazine having di-chloro substituents at side chain of aromatic ring (Table 2; entry 14); **11n** was found to be the least active compound among 2-oxobenzo[1,4]oxazine series. Furthermore, the $-\text{CH}_3$ substituent at benzoxazine nucleus along with electron-donating methyl substituent or halo-substituent at side chain of aromatic ring as in compounds **11g–j**; these were found to show moderate to poor antioxidant activities (Table 2; entry 7–10) in comparison to other analogues of the series.

In the case of 2-oxoquino[1,4]oxalines **14a–h** derivatives, compounds **14c** and **14e** were found to be the best compounds of this series and have shown the antioxidant activities having the IC_{50} value of 9.89 ± 0.15 $\mu\text{g/mL}$ and 8.97 ± 0.13 $\mu\text{g/mL}$, respectively, in comparison to ascorbic acid (Table 2; entry 17 and 19). The 2-oxoquino[1,4]oxalines **14f** having fluoro substituent showed lesser antioxidant activity profile (IC_{50} value of 43.54 ± 0.88 $\mu\text{g/mL}$).

It can be speculated that due to larger electronegativity of fluorine atom, which accumulates the electron density, restricts the delocalization of bonds due to which, the free electrons of **14f** are not easily available for quenching of DPPH radical. Furthermore, extending the side chain of phenyl ring to more electron rich naphthyl ring in **14h** ($\text{IC}_{50} = 14.27 \pm 0.23$ $\mu\text{g/mL}$) showed promising activity. In addition, the un-substituted 2-oxoquino[1,4]oxaline **14a** and dichloro-substituted side chain of aromatic ring having 2-oxo-quino[1,4]oxaline **14d** showed lesser activity (Table 2; entry 15 and 18) in comparison with **14c** and **14e**. The electron-donating substituents at side chain of aromatic ring (compound **14b** and **14g**) showed poor activity profile (Table 2; entry 16 and 21) in comparison with their corresponding halo-substituted analogues **14c** and **14e**.

All the synthesized compounds were also assessed in the ferric to ferrous reduction assay (FRAP assay) taking BHT as standard reference. (Table 2) In the present study, the trend for ferric ion reducing activities of all the compounds **11a–n** and **14a–h**, with respect to standard reference BHT indicates that the seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**) were found more potent antioxidant than BHT. The compound which have the mono-halo (such as: F, Cl, Br) substituent at the side chain of aromatic ring in 2-oxobenzo[1,4]oxazine (compound **11c**, **11e**, **11i**, **11k** and **11l**) exhibited higher antioxidants activity than BHT, whereas 2-oxoquino[1,4]oxaline **14d** and **14h**, which have 2,4-dichloro substituent at side chain of aromatic ring or naphthyl substituent displayed better antioxidant activity than standard reference BHT. Compounds **11a**, **11m**, **14c** and **14f** showed comparable FRAP antioxidant activity than standard reference BHT. Rest of the compounds showed moderate to low FRAP antioxidant activity.

Moreover, for the first time the antioxidant activity of Cephalandole A **16** was also evaluated and found to possess moderate antioxidant activity having IC_{50} value of 11.87 ± 0.14 $\mu\text{g/mL}$ in comparison to ascorbic acid (Table 2; entry 23) in DPPH radical scavenging assay.

These results showed that the mono-halo substitution at side chain of aromatic ring in nitrogen congener of 2-oxobenzo[1,4]oxazines i.e. 2-oxoquino[1,4]oxalines **14c** and **14e** along with un-substituted 2-oxobenzo[1,4]oxazine **11a** were found to be the most active compounds of the series showing promising antioxidant activities in DPPH radical scavenging. Furthermore, in the FRAP antioxidant assay, seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**), which have mono-halo substitution at side chain of aromatic ring in 2-oxobenzo[1,4]oxazine (**11c**, **11e**, **11i** and **11k**, **11l**) and dihalo substituent as well as naphthyl substituent at 2-oxoquino[1,4]oxalines (**14d** and **14h**), showed higher antioxidant activity in comparison with BHT, respectively.

2. 3. Cytotoxicity

Compounds **11a**, **14c**, **14e** and **14h** (which displayed good antioxidant activity in DPPH radical scavenging as-

say) were also assessed for their cytotoxic studies using MTT assay taking 25–250 µg/mL concentration in 3T₃ fibroblast cell lines.²⁷ The screening results showed that these compounds were found non-toxic even at 250 µg/mL and displays allowable values of cell viability. (Figure 3)

3. Experimental Section

3.1. General

All glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on comblap melting point apparatus and are presented uncorrected. Microwave reactor (CEM Discover) was used for operation of reactions. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum 2 spectrophotometer ¹H NMR and ¹³C NMR spectra were recorded on ECS 400 MHz (JEOL) NMR spectrometer using CDCl₃, CD₃OD and CD₃SOCD₃ as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S QToF (Waters, USA) Spectrometer. Column chromatography was performed over Merck silica gel (particle size: 60–120 Mesh) procured from Qualigensä (India), flash silica gel (particle size: 230–400 Mesh). All chemicals and reagents were obtained from Sigma Aldrich (USA), Merck (India) or Spectrochem (India) and were used without further purification.

3.2. General Procedure for the Synthesis of (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11a) in optimization study as given in table 1:

(1) Method A (conventional heating condition):

A solution of **9a** (19.2 mg, 0.10 mmol) and **10a** (10.9 mg, 0.10 mmol) in given solvent (1.0 mL) was heated at given time and temperature (as shown in Table 1). The

progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na₂SO₄ and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired 2-oxobenzo[1,4]oxazine **11a** having good yields (18–67%).

(2) Method B (microwave irradiation condition):

To a solution of **9a** (19.2 mg, 0.10 mmol) in given solvent (1.0 mL) was added **10a** (10.9 mg, 0.10 mmol), and the reaction mixture was irradiated under microwave at given temperature and time (as shown in Table 1). The progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na₂SO₄ and the organic solvent was removed under reduced pressure to give the crude product. The crude product were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired 2-oxobenzo[1,4]oxazine **11a** product having good yields (51–94%).

3.3. General Procedure for the synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ones (11a-n) and (Z)-3-(2-oxophenylethylidene)-3,4-

Figure 3. Percentage cell viability test.

dihydroquinoxalin-2(1H)-ones (14a–h) as given in scheme 1 and scheme 2:

To a solution of compound **9a–f** (0.20 mmol; 1 eq., as given in Scheme 1) or **9a–g** (0.20 mmol; 1 eq. as given in Scheme 2) in diethylene glycol (2.0 mL) was added compound **10a–c** (0.20 mmol; 1 eq., as given in Scheme 1) or **12** and **13** (0.20 mmol; 1 eq., as given in Scheme 2) and the reaction mixture was irradiated under microwave at 150 °C temperature for about 5–10 min depending upon the substrate utilized. The progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na₂SO₄ and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ones **11a–n** and (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-ones **14a–h** having good yields (78–97%).

3. 4. Characterization data of (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ones, (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-ones (11a–n and 14 a–h) and Cephalandole A

Numbering of atoms in ¹H and ¹³C NMR assignment analysis

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11a)^{17a–c}

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 185–186 °C; FT-IR (KBr, ν_{max}/cm⁻¹) 3434, 1754, 1614, 1594, 1270; ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, J = 7.4 Hz, 2H, C4'H, C8'H), 7.55–7.46 (m, 3H, C1'H, C5'H, C7'H), 7.21–7.05 (m, 5H, C5H, C6H, C7H, C8H); ¹³C NMR (100 MHz, CDCl₃) δ 191.6 (>C=O), 156.3 (O=C-O-), 141.3 (C9), 139.1 (C3'), 138.3 (C6'), 132.8 (C3), 128.8 (C10), 127.7 (C4', C8'), 126.0 (C5', C7'), 124.0 (C6), 123.8 (C7), 117.2 (C5), 116.0 (C8), 94.7 (-C=C-); HRMS (ESI) calcd. for C₁₆H₁₁NO₃ [M+H]⁺: 266.0739; found 266.0734.

(Z)-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11b)^{17b,d}

Yellow solid; yield: 95%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 160–162 °C; FT-IR (KBr, ν_{max}/cm⁻¹) 3437, 2925, 1759, 1622, 1110; ¹H NMR (400 MHz, CDCl₃) δ 7.91 (d, J = 7.6 Hz, 2H, C4'H, C8'H), 7.27 (m, 2H, C5'H, C7'H), 7.20–7.16 (m, 2H, C5H, C8H), 7.10–7.06 (m, 2H, C6H, C7H), 7.03 (d, J = 1.2 Hz, 1H, C1'H), 2.41 (s, 3H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 191.4 (>C=O), 156.5 (O=C-O-), 143.6 (C6'), 141.2 (C9), 138.8 (C3'), 135.7 (C3), 129.5 (C10), 127.9 (C4', 8'), 125.9 (C5', C7'), 123.9 (C6), 123.8 (C7), 117.2 (C5), 115.9 (C8), 94.8 (-C=C-), 21.8 (-CH₃); HRMS (ESI) calcd. for C₁₇H₁₃NO₃ [M+H]⁺: 280.0895; found 280.0899.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11c)^{17a,d}

Yellow solid; yield: 90%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 155–157 °C; FT-IR (KBr, ν_{max}/cm⁻¹) 3437, 1759, 1633, 1585, 752; ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, J = 8.4 Hz, 2H, C4'H, C8'H), 7.44 (d, J = 8.4 Hz, 2H, C5'H, C7'H), 7.22–7.09 (m, 4H, C5H, C6H, C7H, C8H), 6.98 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) δ 190.1 (>C=O), 156.1 (O=C-O-), 141.4 (C6'), 139.4 (C9), 139.1 (C3'), 136.6 (C3), 129.1 (C10), 129.0 (C4', C8'), 126.0 (C5', C7'), 124.3 (C6), 123.6 (C7), 117.3 (C5), 116.1 (C8), 94.2 (-C=C-); HRMS (ESI) calcd. for C₁₆H₁₀ClNO₃ [M+2H]⁺: 301.0349; found 301.0345.

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11d)

Yellow solid; yield: 97%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 160–162 °C; FT-IR (KBr, ν_{max}/cm⁻¹) 3436, 1758, 1620, 1577, 1101; ¹H NMR (400 MHz, CDCl₃) δ 12.85 (s, 1H, -NH-), 7.53 (d, J = 7.6 Hz, 1H, C4'H), 7.46 (s, 1H, C8'H), 7.32 (d, J = 8.0 Hz, 1H, C5'H), 7.20–7.12 (m, 5H, C6H, C7H, C8H, C7'H), 6.76 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) 191.1 (>C=O), 155.1 (O=C-O-), 140.8 (C9), 138.4 (C6'), 136.8 (C3'), 136.6 (C4'), 131.8 (C10), 130.0 (C3), 129.9 (C8'), 126.7 (C5'), 125.4 (C7'), 123.9 (C6), 122.7 (C7), 116.6 (C5), 115.6 (C8), 97.5 (C1': -C=C-); HRMS (ESI) calcd. for C₁₆H₉Cl₂NO₃ [M+2H]⁺: 334.9959; found 334.9953.

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11e)^{17e,i}

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 152–154 °C; FT-IR (KBr, ν_{max}/cm⁻¹) 3434, 2925, 1757, 1622, 1596, 1156; ¹H NMR (400 MHz, CDCl₃) δ 8.03 (dd, J = 5.6, 8.8 Hz, 2H, C4'H, C8'H), 7.22–7.11 (m, 6H, C5H, C6H, C7H, C8H, C5'H, C7'H), 7.00 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) δ 190.1 (>C=O), 166.9 (C6'), 156.2 (O=C-O-), 141.3 (C9), 139.2 (C3'), 134.6 (C3), 130.3 (C10), 125.9 (C4', C8'), 124.6 (C6), 123.7 (C7), 117.3 (C5), 116.0 (C5', C7'), 115.8 (C8), 94.3 (C1': -C=C-); HRMS (ESI) calcd. for C₁₆H₁₀FNO₃ [M+H]⁺: 284.0645; found 284.0649.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11f)¹⁷ⁱ

Yellow solid; yield: 93%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 200–202 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3437, 1754, 1624, 1585, 1277, 1111; ¹H NMR (400 MHz, CDCl₃) δ 7.85 (d, J = 8.0 Hz, 2H, C4'H, C8'H), 7.60 (d, J = 8.0 Hz, 2H, C5'H, C7'H), 7.24–7.09 (m, 4H, C5H, C6H, C7H, C8H), 6.97 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) δ 190.3 (>C=O), 156.1 (O=C-O-), 141.4 (C9), 139.4 (C3'), 137.1 (C3), 132.1 (C5', C7'), 129.2 (C10), 127.8 (C4', C8'), 126.0 (C6'), 124.3 (C6), 123.6 (C7), 117.3 (C5), 116.1 (C8), 94.2 (C1': -C=C-); HRMS (ESI) calcd. for C₁₆H₁₀BrNO₃ [M+2H]⁺: 344.9844; found 344.9849.

(Z)-6-methyl-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11g)^{17c}

Yellow solid; yield: 88%, R_f (EtOAc/hexane; 20:80) = 0.90; m.p. 157–158 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3436, 1750, 1618, 1572, 1123, 740; ¹H NMR (400 MHz, CDCl₃) δ 8.00–7.98 (m, 2H, C4'H, C8'H), 7.56–7.45 (m, 3H, C1'H, C5'H, C7'H), 7.07–7.02 (m, 2H, C8H, C6'H), 6.88 (d, J = 8.5 Hz, 2H, C5H, C7H), 2.34 (s, 3H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 191.5 (>C=O), 156.5 (O=C-O-), 139.4 (C9), 139.2 (C3'), 138.4 (C6), 136.1 (C3), 132.7 (C6'), 128.8 (C4', C8'), 127.7 (C5', C7'), 124.8 (C7), 123.4 (C10), 116.8 (C5), 116.2 (C8), 94.5 (C1': -C=C-), 21.08 (-CH₃); HRMS (ESI) calcd. for C₁₇H₁₃NO₃ [M+H]⁺: 280.0895; found 280.0899.

(Z)-6-methyl-3-(2-oxo-2-(p-tolyl) ethylidene)-3, 4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11h)^{17e}

Yellow solid; yield: 89%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 162–164 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3434, 1762, 1602, 1313, 1047; ¹H NMR (400 MHz, CDCl₃) δ 7.90 (d, J = 8.1 Hz, 2H, C4'H, C8'H), 7.27 (d, J = 8.0 Hz, 2H, C5'H, C7'H), 7.06 (d, J = 8.1 Hz, 1H, C8H), 7.02 (s, 1H, C1'H), 6.88 (d, J = 9.4 Hz, 2H, C5H, C7H), 2.42 (s, 3H, C6': -CH₃), 2.35 (s, 3H, C6: -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 191.3 (>C=O), 156.6 (O=C-O-), 143.5 (C6'), 139.3 (C9), 139.0 (C3'), 136.0 (C6), 135.8 (C3), 129.5 (C5', C7'), 127.8 (C4', C8'), 124.6 (C7), 123.5 (C10), 116.8 (C5), 116.1 (C8), 94.6 (C1': -C=C-), 21.7 (C6: -CH₃), 21.0 (C6': -CH₃); HRMS (ESI) calcd. for C₁₈H₁₅NO₃ [M+H]⁺: 294.1052; found 294.1055.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11i)^{17h}

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 145–147 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3437, 1767, 1629, 1582; ¹H NMR (400 MHz, CDCl₃) δ 7.94–7.92 (m, 2H, C4'H, C8'H), 7.46–7.43 (m, 2H, C5'H, C7'H), 7.08 (d, J = 9.2 Hz, 1H, C8H), 6.97 (s, 1H, C1'H), 6.92–6.90 (m, 2H, C5H, C7H), 2.35 (s, 3H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.1 (>C=O), 156.3 (O=C-O-), 139.5 (C9), 139.4 (C6'), 139.0 (C3'), 136.7 (C6), 136.1 (C3), 129.1 (C4', C8'), 129.0 (C5', C7'), 125.0 (C7), 123.2 (C10), 116.9 (C5), 116.3 (C8),

94.1 (C1': -C=C-), 21.1 (-CH₃); HRMS (ESI) calcd. for C₁₇H₁₂ClNO₃ [M+2H]⁺: 315.0506; found 315.0509.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11j)

Yellow solid; yield: 96%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 179–181 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3435, 2923, 1763, 1624, 1543, 1052; ¹H NMR (400 MHz, CDCl₃) δ 7.87–7.84 (m, 2H, C4'H, C8'H), 7.63–7.59 (m, 2H, C5'H, C7'H), 7.08 (d, J = 9.2 Hz, 1H, C8H), 6.96 (s, 1H, C1'H), 6.92–6.90 (m, 2H, C5H, C7H), 2.35 (s, 3H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.2 (>C=O), 156.3 (O=C-O-), 139.5 (C9), 139.4 (C6'), 137.1 (C3'), 136.2 (C6), 132.0 (C3), 129.2 (C5', C7'), 127.7 (C4', C8'), 125.1 (C7), 123.2 (C10), 117.0 (C5), 116.3 (C8), 94.0 (C1': -C=C-), 21.1 (-CH₃); HRMS (ESI) calcd. for C₁₇H₁₂BrNO₃ [M+H]⁺: 358.0001; found 358.0007.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11k)

Yellow solid; yield: 83%, R_f (EtOAc/hexane; 20:80) = 0.70; m.p. 195–197 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3435, 2925, 1759, 1525, 1023; ¹H NMR (400 MHz, CDCl₃) δ 8.03–7.97 (m, 2H, C4'H, C8'H), 7.89–7.86 (m, 2H, C5'H, C7'H), 7.66–7.63 (m, 2H, C5H, C7H), 7.32 (d, J = 9.2 Hz, 1H, C8H), 7.07 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) δ 190.8 (>C=O), 154.9 (O=C-O-), 145.3 (C6), 145.0 (C9), 138.0 (C3'), 136.5 (C3), 132.3 (C5', C7'), 129.4 (C4', C8'), 128.6 (C6'), 124.5 (C10), 119.2 (C7), 118.0 (C5), 111.6 (C8), 96.4 (C1': -C=C-); HRMS (ESI) calcd. for C₁₆H₉BrN₂O₅ [M+2H]⁺: 389.9695; found 389.9699.

(Z)-6-nitro-3-(2-oxo-2-(p-tolyl) ethylidene)-3, 4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11l)

Yellow solid; yield: 81%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 220–223 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3433, 1760, 1624, 1524, 1109; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.73 (d, J = 2.4 Hz, 1H, C4'H), 7.96–7.92 (m, 3H, C5'H, C7'H, C8'H), 7.46–7.37 (m, 3H, C5H, C7H, C8H), 6.95 (s, 1H, C1'H), 2.40 (s, 3H, -CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 189.9 (>C=O), 155.7 (O=C-O-), 145.9 (C6), 143.8 (C9), 139.2 (C6'), 138.5 (C3'), 135.2 (C3), 130.1 (C4', C8'), 128.1 (C5', C7'), 119.5 (C10), 118.9 (C7), 117.7 (C5), 112.6 (C8), 94.8 (C1': -C=C-), 21.7 (-CH₃); HRMS (ESI) calcd. for C₁₇H₁₂N₂O₅ [M+H]⁺: 325.0746; found 325.0741.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11m)

Yellow solid; yield: 78%, R_f (EtOAc/hexane; 20:80) = 0.70; m.p. 239–240 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3435, 2924, 1622, 1525, 1272; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.73 (s, 1H, C5H), 8.03 (d, J = 7.2 Hz, 2H, C4'H, C8'H), 7.91 (d, J = 9.1 Hz, 1H, C7H), 7.59 (d, J = 7.2 Hz, 2H, C5'H, C7'H), 7.42 (d, J = 9.0 Hz, 1H, C8H), 6.90 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 188.9 (>C=O), 156.0 (O=C-O-), 146.0 (C6), 144.7 (C9), 139.8 (C6'), 138.2

(C3'), 137.1 (C3), 129.9 (C4', C8'), 129.6 (C5', C7'), 125.8 (C10), 119.0 (C7), 117.8 (C5), 113.1 (C8), 94.4 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_9ClN_2O_5$ [M+2H]⁺: 346.0200; found 346.0204.

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (11n)

Yellow solid; yield: 80%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 185–187 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3588, 2930, 1769, 1585, 1685, 1108; ¹H NMR (400 MHz, CDCl₃) δ 8.05–7.99 (m, 2H, C7H, C8'H), 7.55 (d, J = 8.4 Hz, 1H, C5'H, C7'H), 7.48 (d, J = 2.0 Hz, 1H, C7'H), 7.37–7.33 (m, 2H, C5H, C8H), 6.89 (s, 1H, C1'H); ¹³C NMR (100 MHz, CDCl₃) δ 192.4 (>C=O), 154.5 (O=C-O-), 145.2 (C6), 145.1 (C9), 138.0 (C6'), 137.6 (C3'), 136.9 (C4'), 132.7 (C3), 131.0 (C8'), 130.8 (C5'), 127.6 (C7'), 124.2 (C10), 119.4 (C7), 118.1 (C5), 111.7 (C8), 100.4 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_8Cl_2N_2O_5$ [M+2H]⁺: 379.9810; found 379.9815.

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14a)^{17a,b}

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 268–269 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3060, 1688, 1619; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.0 (s, 1H, -NH-), 8.07–8.05 (m, 2H, C4'H, C8'H), 7.55–7.48 (m, 3H, C5'H, C6'H, C7'H), 7.21–7.12 (m, 4H, C5H, C6H, C7H, C8H); 7.03 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.9 (>C=O), 155.2 (-NH-C=O), 145.4 (C3), 138.4 (C3'), 131.2 (C6'), 128.2 (C10), 126.5 (C5', C7'), 123.9 (C4', C8'), 123.6 (C9), 123.1 (C6), 116.1 (C7), 115.1 (C5), 114.9 (C8), 89.0 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_{12}N_2O_2$ [M+H]⁺: 265.0899; found 265.0893.

(Z)-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14b)^{17k}

Yellow solid; yield: 96%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 221–222 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3045, 1677, 1615; ¹H NMR (400 MHz, CDCl₃) δ 10.26 (s, 1H, -NH-), 7.96 (d, J = 8.0 Hz, 2H, C4'H, C8'H), 7.31–7.29 (m, 2H, C5'H, C7'H), 7.21–7.12 (m, 4H, C5H, C6H, C7H, C8H); 7.01 (s, 1H, C1'H), 2.43 (s, 3H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 190.5 (>C=O), 158.0 (-NH-C=O), 144.6 (C6'), 142.8 (C3), 136.3 (C3'), 130.3 (C10), 129.4 (C5', C7'), 127.7 (C4', C8'), 125.6 (C9), 124.9 (C6), 123.9 (C7), 116.2 (C5), 115.9 (C8), 90.9 (C1': -C=C-), 21.7 (C6': -CH₃); HRMS (ESI) calcd. for $C_{17}H_{14}N_2O_2$ [M+H]⁺: 279.1055; found 279.1059.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14c)^{17a,k}

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 267–268 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3052, 1686, 1614; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.8 (s, 1H, -NH-), 7.98 (d, J = 8.8 Hz, 2H, C4', C8'), 7.57–7.44 (m, 3H, C5'H, C7'H, C8H), 7.19–7.14 (m, 3H, C5H, C6H, C7H); 6.80

(s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.5 (>C=O), 156.1 (-NH-C=O), 146.7 (C3), 138.1 (C6'), 137.3 (C3'), 129.3 (C10), 127.5 (C4', C8'), 124.8 (C5', C7'), 124.2 (C9), 124.1 (C6), 117.3 (C7), 117.2 (C5), 115.9 (C8), 89.9 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}ClN_2O_2$ [M+2H]⁺: 300.0509; found 300.0503.

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14d)^{17l}

Yellow solid; yield: 95%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 260–262 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3054, 1682, 1618; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.84 (s, 1H, -NH-), 7.63–7.49 (m, 4H, C4'H, C5'H, C7'H, C8H), 7.19–7.15 (m, 3H, C5H, C6H, C7H), 6.44 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.8 (>C=O), 154.8 (-NH-C=O), 145.2 (C6'), 138.2 (C3), 134.7 (C3'), 130.7 (C8'), 130.3 (C4'), 129.2 (C10), 127.1 (C5'), 126.6 (C9), 123.8 (C7'), 123.5 (C6), 123.1 (C7), 116.3 (C5), 114.9 (C8), 92.8 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_{10}Cl_2N_2O_2$ [M+2H]⁺: 334.0119; found 334.0113.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14e)^{17g}

Yellow solid; yield: 93%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 281–282 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3044, 1678, 1606; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.81 (s, 1H, -NH-), 7.92–7.89 (m, 2H, C4'H, C8'H), 7.71–7.69 (m, 2H, C5'H, C7'H), 7.44 (s, 1H, C8H), 7.18–7.14 (m, 3H, C5H, C6H, C7H); 6.79 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 186.5 (>C=O), 155.1 (-NH-C=O), 145.7 (C3), 137.5 (C3'), 131.2 (C5', C7'), 128.5 (C10), 126.5 (C4', C8'), 125.1 (C6'), 123.8 (C9), 123.1 (C6), 116.2 (C7), 115.1 (C5), 114.9 (C8), 88.8 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}BrN_2O_2$ [M+2H]⁺: 344.0004; found 344.0009.

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14f)^{17g}

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 252–253 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3053, 1680, 1614; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.0 (s, 1H, -NH-), 8.02–8.00 (m, 2H, C4'H, C8'H), 7.48 (s, 1H, C8H), 7.33–7.28 (m, 2H, C5'H, C7'H), 7.12–7.11 (m, 3H, C5H, C6H, C7H); 6.76 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.1 (>C=O), 164.4 (C6'), 155.7 (-NH-C=O), 145.7 (C3), 135.3 (C3'), 129.9 (C4', 8'), 129.8 (C10), 126.7 (C9), 124.1 (C6), 123.7 (C7), 116.6 (C5), 113.8 (C8), 115.4 (C5', C7'), 88.9 (C1': -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}FN_2O_2$ [M+H]⁺: 283.0805; found 283.0809.

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14g)^{16e}

Yellow solid; yield: 90%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 241–242 °C; FT-IR (KBr, $\nu_{\max}$ /cm⁻¹) 3058, 1689, 1618; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.95 (s, 1H, -NH-), 7.95 (d, J = 9.2 Hz, 2H, C4'H, C8'H), 7.45–7.44 (m, 1H, C8H), 7.14–7.03 (m, 5H, C5H, C6H, C7H, C5'H, C7'H);

6.77 (s, 1H, C1'H), 3.83 (s, 3H, -OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 187.7 (>C=O), 162.3 (C6'), 155.9 (-NH-C=O), 144.9 (C3), 131.3 (C4', C8', C10), 129.2 (C3'), 126.5 (C9), 124.3 (C6), 123.7 (C7), 116.3 (C5), 115.3 (C8), 113.9 (C5', C7'), 88.9 (C1': -C=C-), 55.4 (-OCH₃); HRMS (ESI) calcd. for C₁₇H₁₄N₂O₃ [M+H]⁺: 295.1004; found 295.1009.

(Z)-3-(2-(naphthalen-2-yl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1H)-one (14h)^{17m}

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 263–264 °C, FT-IR (KBr, ν_{max}/cm⁻¹) 3093, 1694, 1614; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.67 (s, 1H, -NH-), 8.17–8.05 (m, 2H, C4'H, C9'H), 7.81–7.72 (m, 3H, C5'H, C6'H, C8'H), 7.52–7.39 (m, 4H, C6H, C8H, C7'H, C10'H); 7.25–7.14 (m, 2H, C5H, C7H), 6.89 (s, 1H, C1'H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 188.3 (>C=O), 156.3 (-NH-C=O), 146.5 (C3), 144.1 (C3'), 139.9 (C12'), 138.3 (C11'), 129.6 (C9'), 129.4 (C10), 128.5 (C4'), 128.4 (C5'), 127.5 (C9), 127.3 (C8'), 125.3 (C7'), 125.1 (C6'), 124.7 (C6), 124.4 (C7), 117.1 (C10'), 116.3 (C5), 115.9 (C8), 90.1 (C1': -C=C-); HRMS (ESI) calcd. for C₂₀H₁₄N₂O₂ [M+H]⁺: 315.1055; found 315.1059.

Synthesis of Cephalandole A (16): To a solution of 3-Indoleglyoxylic acid **15** (226.9 mg, 1.20 mmol) in diethylene glycol was added **10a** (130.8 mg, 1.20 mmol) and the reaction mixture was irradiated under MW at 150 °C temperature for 10 min and the progress of reaction was monitored by TLC. After that, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na₂SO₄ and the organic solvent was removed under reduced pressure to give the crude product. The crude product was further purified by flash column chromatography method over silica gel using hexane/ethyl acetate (8:2; v/v) as an eluent which afforded the pure desired Cephalandole A **16** having good yield (280.5 mg, 89%). Yellowish solid; m.p. 232–233 °C; ¹H NMR^{24c} (400 MHz, DMSO-*d*₆) δ 11.98 (s, 1H), 8.76–8.74 (m, 1H), 8.69 (s, 1H), 7.85 (d, J = 6.4 Hz, 1H), 7.54–7.39 (m, 4H), 7.27–7.25 (m, 2H); HRMS (ESI) calcd. for C₁₆H₁₀N₂O₂ [M+H]⁺: 263.0742; ; found 263.0749.

4. Supplementary Data

The characterization spectra of synthesized 2-oxobenzo[1,4]oxazines **11a–n**, 2-oxoquino[1,4]oxalines **14a–h** and Cephalandole A (**16**) are provided in supplementary material via the “Supplementary Content” section of this article's webpage.

5. Conclusions

In summary, we have developed a simple and highly efficient MW-assisted protocol for the synthesis of func-

tionalized 2-oxobenzo[1,4]oxazines **11a–n** and 2-oxoquino[1,4]oxalines **14a–h** in excellent yields. This reaction tolerates a broad range of substrates, and provides a straightforward access to functionalized 2-oxobenzo[1,4]oxazines and 2-oxoquino[1,4]oxalines. The practical applicability of developed methodology was confirmed by the gram scale synthesis of **11a**, **14c** and **14e**, along with the synthesis of Cephalandole A (**16**) (89% yield). All the synthesized compounds were screened for their *in vitro* antioxidant activities using DPPH radical scavenging and FRAP assays. Compounds **11a**, **14c** and **14e**, the most active compounds of the series, were found to show IC₅₀ value of **10.20 ± 0.08** µg/mL, **9.89 ± 0.15** µg/mL and **8.97 ± 0.13** µg/mL, respectively as compared to standard reference ascorbic acid (IC₅₀ = 4.57 µg/mL) in DPPH assay, whereas in FRAP assay, seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**) exhibited higher antioxidant activity in comparison with BHT. Cytotoxic studies revealed that the non-toxic nature of compounds **11a**, **14c**, **14e** and **14h** even at 250 µg/mL concentration. To the best of our knowledge, this is the first report of microwave-assisted synthesis and *in vitro* antioxidant activities of functionalized 2-oxobenzo[1,4]oxazines **11a–n**; and 2-oxoquino[1,4]oxalines **14a–h** and Cephalandole A **16** in excellent yields. The potential *in vitro* antioxidant activity combined with ease of preparation qualifies these compounds as candidates for further lead optimization studies.

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Povzetek

V prispevku je opisana okolju prijazna in s pomočjo mikrovalov spodbujena sinteza funkcionaliziranih (Z)-3-(2-okso-2-feniletiliden)-3, 4-dihidro-2H-benzo[b][1,4]oksazin-2-onov (**11a–n**) z odličnimi izkoristki (do 97%) in (Z)-3-(2-okso-2-feniletiliden)-3,4-dihidrokinoksalin-2(1H)-onov (**14a**) (do 96% izkoristek). Uporabna vrednost razvite metodologije je prikazana na sintezi spojin **11a**, **14c** in **14e** na gramski skali ter na sintezi protitumorskega alkaloida cefalandola A (**16**, 89% izkoristek). Vse sintetizirane spojine, **11a–n**, **14a–h** in **16**, so bile preizkušene na *in vitro* antioksidativno aktivnost, DPPH radikalsko lovljenje in FRAP test. Pri DPPH testiranju so se kot najbolj aktivne pokazale spojine **11a**, **14c** in **14e** z IC_{50} vrednostmi 10.20 ± 0.08 $\mu\text{g/mL}$, 9.89 ± 0.15 $\mu\text{g/mL}$ in 8.97 ± 0.13 $\mu\text{g/mL}$ glede na standardno referenco (askorbinska kislina, $IC_{50} = 4.57$ $\mu\text{g/mL}$), medtem, ko je pri FRAP antioksidativnem testu sedem spojin (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** in **14h**) izkazalo višjo antioksidativno aktivnost kot referenčni standard BHT ($C_{0.5\text{FRAP}} = 546.2$ μM). Poleg tega so študije citotoksičnosti spojin **11a**, **14c**, **14e** in **14h** hh pri MTT testiranju pokazale, da so te spojine netoksične do celičnih linij naravnih 3T₃ fibroblastov.