ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2015, Vol. 41, No. 5, pp. 554–561. © Pleiades Publishing, Ltd., 2015.

Synthesis and Biological Efficacy of Novel Piperazine Analogues Bearing Quinoline and Pyridine Moieties[1]

M. Al-Ghorbani[a], N. D. Rekha[b], V. Lakshmi Ranganatha[a, c], T. Prashanth[a, d], T. Veerabasappagowda[b], and S. A. Khanum[a, 2]

[a] *Department of Chemistry, Yuvarajas College, University of Mysore, Mysore, Karnataka, India*
[b] *Department of Studies in Biotechnology, JSS College of Arts, Commerce and Science, Mysore, Karnataka, India*
[c] *PG Department of Chemistry, St. Philomena's College, Bannimantap, Mysore - 570015*
[d] *Department of Chemistry, The National Institute of Engineering, Manandavadi Road, Mysore 570008*

Received August 24, 2014; in final form, January 12, 2015

Abstract—A series of novel piperazine analogues bearing quinolin-8-yloxy-butan-1-ones/pyridin-2-yloxy-ethanones were synthesized by a simple and convenient approach based on various substituted piperazine incorporating quinoline and pyridine moieties. The analogues were evaluated for in vitro antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous ion radical scavenging activities and anti-inflammatory activity by inhibition of *Vipera russelli* venom (PLA_2) and gastric K^+/H^+-ATPase activities. Most of the title compounds exhibited promising activity. Best antioxidant and PLA_2-inhibiting activities were found for piperazine analogues with phenyl and nitro phenyl groups, whereas methoxy group on phenyl piperazine indicated selectivity for the H^+/K^+-ATPase.

Keywords: piperazine analogues, antioxidant, PLA_2, H^+/K^+-ATPase
DOI: 10.1134/S1068162015040020

INTRODUCTION

Antioxidant therapies are gaining importance due to their ability to retard disease progression by reducing the damage caused by free radical oxidative stress in a patient [1]. Physiological levels of reactive oxygen species (ROS) play a vital role as signaling molecules to mediate numerous biological functions causing alterations in cell growth, gene expression, and host defense [2]. Under inflammatory conditions, the presence of excess ROS ($O_2^{\bullet -}$, $^{\bullet}OH$, H_2O_2, $NO^{\bullet}$, $ONOO^-$) can initiate damage to nucleic acids, proteins, carbohydrates, and lipids in many types of cells, including macrophages [3].

The association of antioxidants with inflammation stems from the recognition that free radicals are produced during the inflammatory process by macrophages. It has been reported that ROS are involved in the cyclooxygenase- and lipoxygenase-mediated conversion of arachidonic acid into proinflammatory intermediates and possess the ability to activate PLA_2 enzyme, which further augments the inflammatory process and worsens the disease during chronic inflammatory conditions [4]. Therefore, suppression of these pro-inflammatory lipid mediators in conjunction with free radicals has long been considered in the therapeutic process of various inflammatory disorders.

On the other hand, the inhibition of gastric acid secretion, especially by antagonism of the H2 receptor, has been proven a powerful tool in the treatment of gastric and duodenal ulcer diseases [5]. Recently, agents that completely suppress acid secretion by inhibition of the gastric proton pump H^+/K^+-ATPase have been identified.

Piperazine and their derivatives have been found to exhibit a variety of biological activities and are well known for their medicinal importance and recognized for their use as antidepressive [6], antihistamine [7], anticancer [8–10], and antioxidant [11] activities.

The incorporation of synthetic piperazine is an important strategy in drug discovery due to its easy modifiability, proper alkality, water solubility, and the capacity for the formation of hydrogen bonds and adjustment of molecular physicochemical properties [12]. The piperazine nucleus incorporated the quinoline and pyridine moiety into a rigid framework. It has been reported that quinoline and pyridine derivatives possess appreciable anti-inflammatory activity [13]. Moreover, piperazine and related derivatives have shown special ability to scavenge ROS in processes involving free radical injury [14] and are effective as reversible inhibitors of gastric H^+/K^+-ATPase [15].

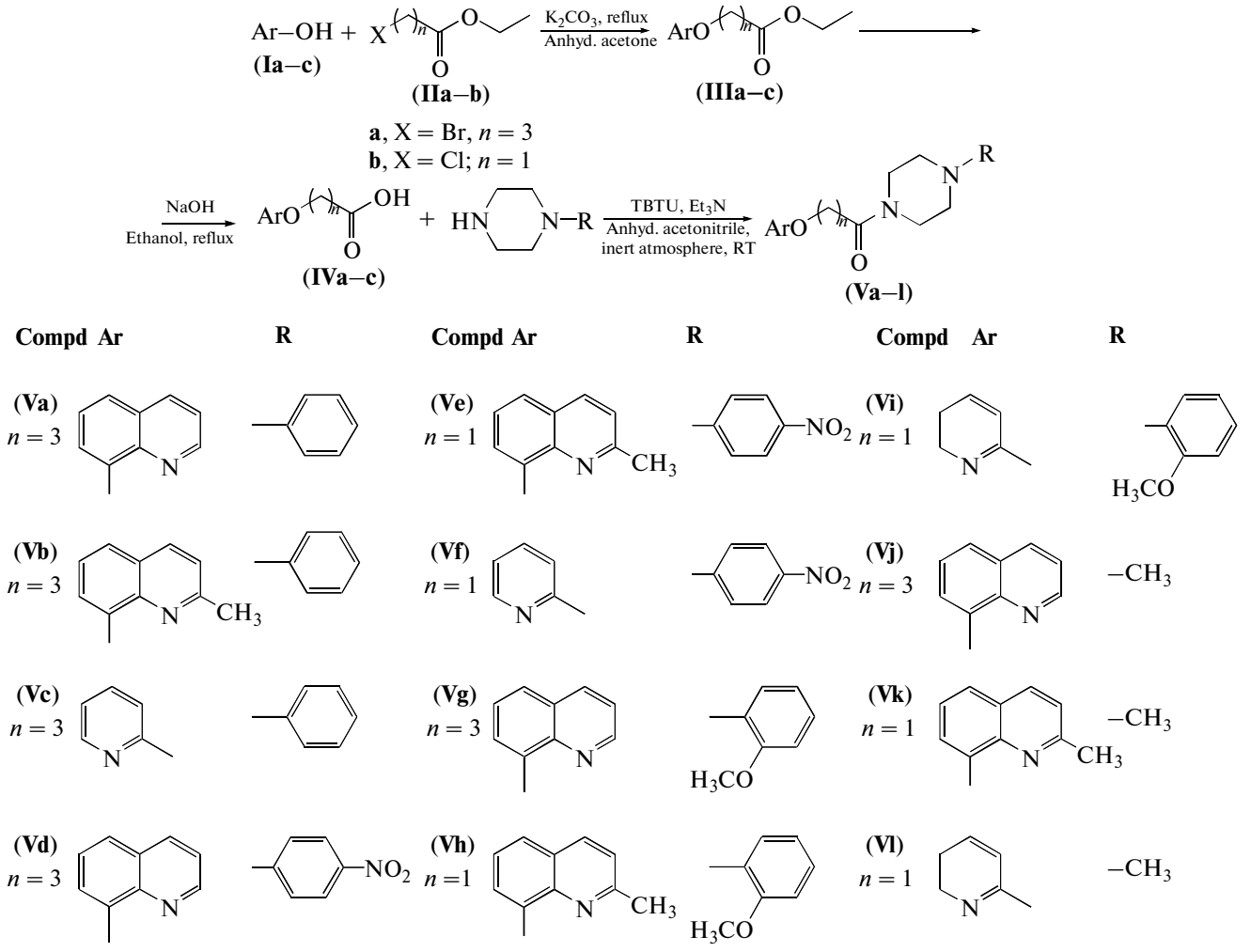
[1] The article is published in the original.

[2] Corresponding author: phone: +9901888755; fax: +821 2419239; e-mail: shaukathara@yahoo.co.in.

Based on the above information and continuing our research program aimed at developing simple and efficient synthesis of pharmacologically useful heterocyclic analogues, we envisage that incorporating quinoline and pyridine cores in substituted piperazine frame could lead to the potent antioxidant activity, as well as PLA_2 and H^+/K^+-ATPase inhibition activities.

RESULTS AND DISCUSSION

In this study, novel derivatives of piperazine analogues were synthesized in an attempt to find new compounds having antioxidant activity and anti-inflammatory related activities, such as inhibition of PLA_2 and gastric H^+/K^+-ATPase activity. The synthetic routes for the newly synthesized piperazine analogues (**Va–l**) are illustrated and outlined in the Scheme 1. The structure elucidations of the newly synthesized compounds were confirmed by ^1H and ^{13}C NMR, mass spectrometry, and elemental analysis. In the ^1H NMR spectra of (**Va–l**), two sets of multiplet signals at δ 3.1–4.0 ppm each belonging to the four protons of two CH_2 groups in piperazine ring were observed. The ^1H NMR spectrum of compound (**Va**) showed the multiplet signals in the region of δ 6.8–7.3 ppm, which appeared due to phenyl protons. ^{13}C NMR spectra also confirmed the piperazine structure of (**Va–l**) due to peaks appearance in the range δ 44.08–67.89. The mass spectra of compounds (**Va–l**) were in agreement with their assigned structures. The mass spectrum of (**Va**) showed molecular ion peak at 376 (M + 1), which corresponds to the molecular formula $C_{23}H_{25}N_3O_2$. Similarly, the spectral values for all the compounds and C, H, N analyses are given in the experimental part.



Scheme 1. Synthesis of piperazine analogues (**Va–l**).

In vitro antioxidant activities were measured against DPPH and ferrous ion chelating radicals. DPPH radical scavenging activity evaluation is a rapid and convenient technique for screening the antioxi-

Table 1. DPPH radical scavenging activity of the compounds (**Va–l**)*

Test samples	Concentration, μg/mL					
	2	4	6	8	10	IC_{50}, μg/mL
(**Va**)	23.74	34.23	45.47	57.45	77.53	6.7
(**Vb**)	29.72	45.64	59.62	73.71	99.4	4.6
(**Vc**)	36.63	48.48	67.1	79.65	92.65	4.1
(**Vd**)	22.73	45.51	52.25	74.18	87.3	5.2
(**Ve**)	26.4	47.27	65.61	78.29	90.17	4.4
(**Vf**)	38.31	53.46	71.12	82.35	92.52	3.5
(**Vg**)	30.14	42.57	58.3	75.03	95.24	5.1
(**Vh**)	21.52	41.59	64.61	84.72	98.55	4.7
(**Vi**)	24.59	43.74	67.66	86.51	98.58	4.5
(**Vj**)	33.39	46.58	66.78	77.58	84.22	4.4
(**Vk**)	39.71	51.71	65.64	83.22	97.52	3.8
(**Vl**)	29.56	41.31	63.62	79.14	93.86	4.6
BHT	34.86	48.46	65.48	78.6	90.46	4.4

* Results presented here are the mean values from three independent experiments.

Table 2. Ferrous ion radical scavenging activity of the compounds (**Va–l**)*

Test samples	Concentration, μg/mL					
	4	8	12	16	20	IC_{50}, μg/mL
(**Va**)	28.5	48.82	69.59	83.58	94.7	9.5
(**Vb**)	33.28	57.45	67.89	79.44	88.13	7.6
(**Vc**)	23.6	46.56	64.5	78.51	94.43	9.1
(**Vd**)	19.64	37.64	55.47	72.48	89.14	10.9
(**Ve**)	31.46	43.44	66.44	78.12	90.34	9.7
(**Vf**)	24.57	48.48	65.45	78.35	91.5	8.6
(**Vg**)	30.54	42.24	57.47	70.42	83.59	10.6
(**Vi**)	32.65	41.92	54.83	76.12	94.51	11.2
(**Vj**)	34.49	56.17	74.88	82.84	94.86	6.2
(**Vl**)	21.24	41.29	63.62	81.73	92.54	9.3
BHT	22.65	43.5	64.57	86.62	93.58	9.6

* Results presented here are the mean values from three independent experiments.

dant activities. It can be seen from Table 1 that, in DPPH assay, compounds (**Vc**), (**Vf**), and (**Vk**) with IC_{50} values of 4.1, 3.5, and 3.8 μg/mL, respectively, showed better radical scavenging activities than the synthetic commercial antioxidant BHT with the highest scavenging of 92–97%. Compounds (**Ve**), (**Vi**), (**Vj**), and (**Vl**) with IC_{50} values of 4.4, 4.5, 4.4, and 4.6 μg/mL displayed good DPPH radical scavenging activity. Compounds (**Vb**), (**Vd**), (**Vg**), and (**Vh**) showed moderate radical scavenging abilities, while compound (**Va**) showed poor radical scavenging ability in comparison with the standard.

Metal chelating capacity is important since it reduces the concentration of the catalyzing transition metal in lipid peroxidation (thus delaying metal-catalyzed oxidation) [16]. Since ferrous ions constitute the most effective pro-oxidants in food and biological systems, the good chelating effect and removal of free iron from circulation would be beneficial and correct approach to prevent oxidative stress-induced disorder. From the results of ferrous ion chelating assay (Table 2), the compounds (**Vb**), (**Vc**), (**Vf**), (**Vj**), and (**Vl**) with IC_{50} values of 7.65, 9.12, 8.60, 6.23, and 9.33 μg/mL, respectively, showed higher radical scavenging activities than that of the standard BHT. Other compounds

Table 3. PLA_2 inhibition activity of compounds (**Va–l**)

Test samples	Concentration, μg/mL						
	10	20	30	40	50	60	IC_{50}, μg/mL
(**Va**)	12.38	37.9	54.37	66.74	78.33	95.63	27.5
(**Vb**)	24.26	40.04	56.59	68.53	79.03	93.61	26
(**Vc**)	26.74	37.24	46.94	58.13	75.73	89.03	32.4
(**Vd**)	25.33	34.92	54.42	68.88	79.52	92.61	29
(**Ve**)	18.91	25.43	47.68	59.31	70.15	81.95	32.3
(**Vf**)	10.36	28.85	47.74	56.74	75.73	84.72	31
(**Vg**)	8.04	17.44	36.94	52.13	65.63	75.33	38
(**Vh**)	5.31	28.5	45.7	63.22	76.3	89.53	32
(**Vi**)	17.3	29.89	44.38	52.87	65.77	79.36	40
(**Vj**)	11.36	29.15	47.54	65.53	73.62	81.71	31.2
(**Vk**)	74.84	67.5	60.06	52.91	45.77	38.43	Inactive
(**Vl**)	12.37	10.36	8.75	6.94	4.72	2.61	Inactive
Control	100	–	–	–	–	–	5

exhibited good to moderate radical scavenging activity; compounds (**Vh**) and (**Vk**) failed to scavenge ferrous ion radical. Compounds (**Vb**), (**Vc**), (**Vf**), (**Vj**), and (**Vl**) demonstrated a marked capacity for iron binding, suggesting their role as hydroxyl radical protector. The results reveal that phenyl and nitrophenyl groups in the piperazine ring of (**Vb**), (**Vc**), and (**Vf**), as well as compounds (**Vj**), (**Vk**), and (**Vl**) with the methyl group in the piperazine, possess potent DPPH and ferrous ion radical scavenging activities. However, with few exceptions, it has been noted that phenyl, nitrophenyl and *N*-methyl piperazine were found to be more suitable for reduction of DPPH [14] and ferrous ion chelating.

The Russell viper venom PLA_2 is known to induce presynaptic neurotoxicity and myotoxicity in experimental mice [17]. All these maladies are associated with PLA_2 activity. Compounds (**Va–l**) were tested for in vitro anti-inflammatory properties by studying their indirect hemolytic PLA_2 activity (Table 3). Compounds (**Va**), (**Vb**), and (**Vd**) having phenyl and nitrophenyl substituents in the piperazine ring inhibited snake venom PLA_2 in a dose dependent manner with IC_{50} values of 27.5, 26, and 29 μg/mL respectively, while compounds (**Vk**) and (**Vl**) having methyl substituent in the piperazine ring failed to inhibit the snake venom PLA_2. On the other hand, the rest of the compounds, (**Vc**), (**Ve**), (**Vf**), (**Vh**), and (**Vj**) with IC_{50} values of 32.4, 32.3, 31, 32, and 31.2 μg/mL, respectively, showed moderate activity and compounds (**Vg**) and (**Vi**) with IC_{50} values of 38 and 40 μg/mL, respectively, showed low activity. Therefore, compounds (**Va**), (**Vb**), and (**Vd**) are promising anti-inflammatory molecules compared to other substances and they tempt to suggest a role as a candidate for the treatment of snake bite.

Compounds (**Va–l**) were tested for their ability to inhibit gastric H^+/K^+-ATPase (Table 3). Omeprazole was used as the reference compound. Several compounds exhibited potent inhibitory activity on H^+/K^+-ATPase. Compounds (**Vc**), (**Vg**), (**Vh**), and (**Vi**) having phenyl and methoxyphenyl substituent in the piperazine ring with IC_{50} values of 5.2, 4.5, 4.9, and 5.3 μg/mL, respectively, indicated selectivity for the H^+/K^+-ATPase. IC_{50} values for compounds (**Vb**), (**Ve**), and (**Vj**) could not be calculated as they had no inhibitory activity. Compound (**Vg**) was found to be the most active among the tested compounds.

From the PLA_2 and ATPase results point of view, it can be pointed out that phenyl and nitrophenyl substituents on the piperazine ring showed promising venom PLA_2 inhibition while methoxyphenyl substituent in the piperazine ring was found to exhibit the significant inhibition against H^+/K^+-ATPase. Further modification of side chain quinoline, particularly the presence of the electron donating methyl group or stereo chemical factors due to ortho substitution, might be the cause for failed activity associated with the compounds (**Vh**) and (**Vk**) in ferrous ion, (**Vk**) in PLA_2, and (**Vb**) and (**Ve**) in H^+/K^+-ATPase assays. It is noteworthy that compounds (**Vc**) and (**Vf**) having a pyridine ring with phenyl and nitrophenyl groups, respectively, showed potent inhibition in all the assays.

EXPERIMENTAL

All the chemicals and reagents were purchased from Sigma Aldrich Chemical Pvt Ltd. All the solvents were double distilled before use. *Vipera russelli* venom was obtained from IRULA snake catchers, Madras, India. Melting points were determined in open capillary tubes on an electrothermal melting point appara-

tus. Absorbance was measured on a U-2900 (Hitachi) UV-VIS spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were registered on an API-4000LC-MS/MS apparatus. The elemental analysis of the compounds was performed on a Perkin Elmer 2400 Elemental Analyzer. The results of elemental analyses were within ±0.4% of the theoretical values.

Synthesis of Compounds

The reaction sequence for different title compounds (**Va**–**l**) is outlined in the Scheme 1. The starting substituted hydroxyl compounds (**Ia**–**c**) were reacted with (**IIa**–**b**) in the presence of anhydrous potassium carbonate and dry acetone at reflux temperature to afford compounds (**IIIa–c**) [18]. These materials were subjected to hydrolysis using NaOH solution in ethanol to afford the product (**IVa**–**c**). Finally, the target piperazine analogues (**Va**–**l**) were obtained in good yields by stirring of (**IVa**–**c**) with substituted piperazines in the presence of *N,N,N',N'*-tetramethyl-*o*-(benzotriazol-1-yl) uroniumtetrafluoroborate (TBTU) as coupling reagent and triethylamine in dry acetonitrile at RT under inert atmosphere.

General synthetic procedure for ethyl butyrate and ethyl acetate analogues (IIIa–c). Synthetic procedure for compounds (**IIIa–c**) has been reported previously [18, 19].

4-(Quinolin-8-yloxy)-butyric acid ethyl ester (IIIa). mp 62–64°C (ref. 60°C [19]).

General synthetic procedure for butyric acid and acetic acid analogues (IVa–c). Compounds (**IIIa–c**) (0.02 mol) were dissolved in ethanol (15 mL), sodium hydroxide (0.035 mol) in water (5 mL) was added, and the mixture was refluxed for 5–9 h. The reaction mixture was cooled and acidified with 2 N hydrochloric acid. The precipitate was filtered, washed with water, and finally recrystallized from methanol to afford desired compounds (**IVa**–**c**). Compound (**IVa**) is taken as a representative example to explain physical and characterization data.

4-(Quinolin-8-yloxy)-butyric acid (IVa). Yield 80%, pink solid, mp 168–170°C. ^{1}H NMR: 2.1 (m, 2H, CH_2), 2.6 (t, 2H, $COCH_2$), 4.2 (t, 2H, OCH_2), 7.1–8.8 (m, 6H, quinoline Ar-H), 12.5 (s, 1H, COOH). LC-MS: *m/z* 232 (*M* + 1). Anal. calcd. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.46; H, 5.53; N, 5.88%.

General synthetic procedure for piperazine analogues (Va–l). To the mixture of compounds (**IVa**–**c**) (2 mmol) in dry acetonitrile (15 mL), triethylamine (3 mmol) followed by substituted piperazines (2 mmol) was added. The mixture was stirred for 30 min and TBTU (2mmol) was then added and stirring was continued at room temperature under an inert atmosphere for 10–24 h; the completion of the reaction was monitored by TLC using chloroform–methanol (9 : 1) as eluent. The solvent was evaporated at reduced pressure, quenched by the addition of cold water (20 mL), and the obtained solids (**Va**), (**Vb**), (**Vc**), (**Vd**), (**Ve**), (**Vf**), (**Vi**), and (**Vk**) were filtered, dried, and recrystallized from ethanol. In contrast, compounds (**Vg**), (**Vh**), (**Vj**), and (**Vl**) were obtained by extracting with ethyl acetate. The extract was washed successively with a solution of 10% HCl (20 mL), 10% $NaHCO_3$ (20 mL), and water (20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated, the crude product was purified by column chromatography (eluent: hexane–dichloromethane–acetone, 5 : 3 : 2) to achieve pure piperazine derivatives. The physical and analytical data of the synthesized title compounds (**Va**–**l**) are given below.

1-[4-(Phenyl)-piperazin-1-yl]-4-(quinolin-8-yloxy)-butan-1-one (Va). Yield 78%, white solid, mp112–114°C. ^{1}H NMR: 2.4 (m, 2H, CH_2), 2.7 (t, 2H, $COCH_2$), 3.1 (m, 4H, piperazine), 3.7 (m, 4H, piperazine), 4.4 (t, 2H, OCH_2), 7.1–8.9 (m, 6H, quinoline Ar-H), 6.8–7.3 (m, 5H, Ar-H). ^{13}C NMR: 24.41, 29.57, 40.47, 44.25, 46.42, 49.00, 67.58, 76.82, 77.21, 77.26, 109.23, 112.57, 119.69, 122.77, 125.64, 126.47, 129.24, 136.32, 139.85, 140.87, 149.39, 154.65, 171.46. LC-MS: *m/z* 376 (*M* + 1). Anal. calcd. for $C_{23}H_{25}N_3O_2$: C, 73.57; H, 6.71; N, 11.19. Found: C, 73.45; H, 6.54; N, 11.35%.

1-(4-Phenyl-piperazin-1-yl)-2-(2-methyl-quinolin-8-yloxy)-ethanone (Vb). Yield 76%, white solid, mp 137–139°C. ^{1}H NMR: 3.2 (s, 3H, quinoline CH_3), 3.5 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.9 (s, 2H, OCH_2CO), 6.9–8.4 (m, 5H, quinoline Ar-H), 6.8–7.3 (m, 5H, Ar-H). ^{13}C NMR: 24.45, 40.18, 44.54, 46.27, 49.28, 67.41, 76.00, 76.76, 77.87, 109.42, 112.70, 119.95, 121.86, 126.56, 126.89, 129.70, 136.17, 138.66, 140.95, 149.39, 154.48, 171.87. LC-MS: *m/z* 362 (*M* + 1). Anal. calcd. for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.20; H, 6.19; N, 11.56%.

1-(4-Phenyl-piperazin-1-yl)-2-(pyridin-2-yloxy)-ethanone (Vc). Yield 64%, white solid, mp 142–144°C. ^{1}H NMR: 3.3 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.8 (t, 2H, OCH_2), 6.2–7.2 (m, 4H, pyridine Ar-H), 6.9–7.4 (m, 5H, Ar-H). ^{13}C NMR: 40.56, 44.15, 46.07, 49.45, 67.89, 76.20, 77.01, 77.33, 109.29, 112.78, 119.75, 124.47, 136.83, 139.42, 146.32, 151.42, 171.40. LC-MS: *m/z* 298 (*M* + 1). Anal. calcd. for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.53; H, 6.12; N, 14.27%.

1-[4-(4-Nitro-phenyl)-piperazin-1-yl]-4-(quinolin-8-yloxy)-butan-1-one (Vd). Yield 80%, yellow solid, mp 115–117°C.^{1}H NMR: 2.5 (m, 2H, CH_2), 2.8 (t, 2H, $COCH_2$), 3.5 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.5 (t, 2H, OCH_2), 7.1–8.9 (m, 6H, quinoline Ar-H), 6.7–7.6 (m, 4H, Ar-H). ^{13}C NMR: 24.51, 29.41, 40.86, 44.62, 46.86, 49.13, 67.77, 76.75, 77.01,

77.26, 109.09, 112.81, 119.75, 121.61, 125.92, 126.83, 129.73, 136.04, 139.04, 144.35, 149.19, 154.35, 171.26. LC-MS: *m/z* 421 (*M* + 1). Anal. calcd. for $C_{23}H_{24}N_4O_4$: C, 65.70; H, 5.75; N, 13.33. Found: C, 65.78; H, 5.89; N, 13.17%.

1-[4-(4-Nitro-phenyl)-piperazin-1-yl]-2-(2-methyl-quinolin-8-yloxy)-ethanon (Ve). Yield 72%, yellow solid, mp 118–120°C. ^{1}H NMR: 3.3 (s, 3H, quinoline CH_3), 3.5 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.8 (s, 2H, OCH_2CO), 7.0–8.5 (m, 5H, quinoline Ar-H), 6.7–7.6 (m, 4H, Ar-H). ^{13}C NMR: 25.75, 40.25, 44.11, 46.75, 49.73, 67.89, 76.80, 77.38, 77.46, 109.45, 112.70, 119.45, 121.28, 125.74, 126.57, 129.29, 136.04, 137.77, 144.61, 149.40, 154.78, 171.76. LC-MS: *m/z* 407 (*M* + 1). Anal. calcd. for $C_{22}H_{22}N_4O_4$: C, 65.01; H, 5.46; N, 13.78. Found: C, 65.16; H, 5.30; N, 13.62%.

1-[4-(4-Nitro-phenyl)-piperazin-1-yl]-2-(pyridin-2-yloxy)-ethanone (Vf). Yield 75%, brown solid, mp 184–186°C. ^{1}H NMR: 3.3 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.7 (t, 2H, OCH_2), 6.2–7.2 (m, 4H, pyridine Ar-H), 6.9–7.4 (m, 4H, Ar-H). ^{13}C NMR: 40.09, 44.59, 46.97, 49.68, 67.25, 76.50, 77.87, 77.30, 109.09, 112.79, 119.06, 124.33, 136.65, 139.34, 146.07, 151.65, 171.16. LC-MS: *m/z* 343 (*M* + 1). Anal. calcd. for $C_{17}H_{18}N_4O_4$: C, 59.64; H, 5.70; N, 16.37. Found: C, 59.61; H, 5.99; N, 16.13%.

1-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-4-(quinolin-8-yloxy)-butan-1-one (Vg). Yield 72%, pale yellow oil. ^{1}H NMR: 2.4 (m, 2H, CH_2), 2.8 (t, 2H, $COCH_2$), 3.4 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.2 (s, 3H, OCH_3), 4.6 (t, 2H, OCH_2), 7.0–8.9 (m, 6H, quinoline Ar-H), 6.6–7.2 (m, 4H, Ar-H). ^{13}C NMR: 24.36, 29.19, 40.36, 44.29, 46.56, 49.17, 56.5, 67.35, 76.43, 77.22, 77.21, 109.37, 112.78, 119.90, 121.69, 125.73, 126.36, 129.62, 136.37, 139.61, 140.35, 149.28, 154.39, 171.72. LC-MS: *m/z* 406 (*M* + 1). Anal. calcd. for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.97; H, 6.47; N, 10.25%.

1-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-2-(2-methyl-quinolin-8-yloxy)-ethanone (Vh). Yield 58%, yellow solid, mp 106–108°C. ^{1}H NMR: 3.3 (s, 3H, quinoline CH_3), 3.5 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.1 (s, 3H, OCH_3), 4.8 (s, 2H, OCH_2CO), 6.8–8.4 (m, 5H, quinoline Ar-H), 6.6–7.2 (m, 4H, Ar-H). ^{13}C NMR: 25.82, 40.27, 44.29, 46.92, 49.76, 56.5, 67.24, 76.13, 77.43, 75.98, 109.33, 112.94, 119.74, 121.61, 125.21, 126.61, 129.13, 135.12, 139.79, 144.41, 149.19, 154.80, 169.22. LC-MS: *m/z* 392 (*M* + 1). Anal. calcd. for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.69; H, 6.23; N, 10.92%.

1-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-2-(pyridin-2-yloxy)-ethanone (Vi). Yield 66%, yellow solid, mp 155–157°C. ^{1}H NMR: 3.3 (m, 4H, piperazine), 4.0 (m, 4H, piperazine), 4.3 (s, 3H, OCH_3), 4.9 (t, 2H, OCH_2), 6.2–7.0 (m, 4H, pyridine Ar-H), 6.7–7.4 (m, 4H, Ar-H). ^{13}C NMR: 40.45, 44.15, 46.58, 49.31, 56.5, 67.45, 76.22, 77.01, 77.78, 109.70, 112.18, 119.65, 124.83, 136.83, 139.02 146.70, 151.18, 171.65. LC-MS: *m/z* 328 (*M* + 1). Anal. calcd. for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.28; H, 6.09; N, 12.50%.

1-(4-Methyl-piperazin-1-yl)-4-(quinolin-8-yloxy)-butan-1-one (Vj). Yield 65%, dark yellow oil. ^{1}H NMR: 2.2 (m, 2H, CH_2), 2.7 (t, 2H, $COCH_2$), 3.7 (m, 4H, piperazine), 3.6 (s, 3H, CH_3), 4.0 (m, 4H, piperazine), 4.5 (t, 2H, OCH_2), 6.9–8.8 (m, 6H, quinoline Ar-H). ^{13}C NMR: 24.13, 29.60, 40.75, 44.40, 46.48, 48.50, 49.26, 67.43, 76.27, 77.01, 105.76, 109.45, 113.12, 139.24, 140.35, 149.46, 154.24, 171.63. LC-MS: *m/z* 314 (*M* + 1). Anal. Cal. for $C_{18}H_{23}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 69.18; H, 7.28; N, 13.25%.

1-(4-Methyl-piperazin-1-yl)-2-(2-methyl-quinolin-8-yloxy)-ethanone (Vk). Yield 65%, brown solid, mp 123–125°C. ^{1}H NMR: 3.2 (s, 3H, quinoline CH_3), 3.5 (s, 3H, CH_3), 3.7 (m, 4H, piperazine), 4.0 (m, 4H, piperazine), 4.7 (s, 2H, OCH_2CO), 6.7–8.3 (m, 5H, quinoline Ar-H). ^{13}C NMR: 25.25, 40.03, 44.34, 46.62, 48.09, 49.74, 67.89, 76.75, 77.71, 100.26, 109.63, 112.17, 139.57, 140.80, 149.65, 154.87, 171.09. LC-MS: *m/z* 300 (*M* + 1). Anal. calcd. for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07; N, 14.04. Found: C, 68.01; H, 7.32; N, 13.91%.

1-(4-Methyl-piperazin-1-yl)-2-(pyridin-2-yloxy)-ethanone (Vl). Yield 60%, dark yellow oil. ^{1}H NMR: 3.4 (s, 3H, CH_3), 3.9 (m, 4H, piperazine), 3.9 (m, 4H, piperazine), 4.9 (t, 2H, OCH_2), 6.2–7.4 (m, 4H, pyridine Ar-H). ^{13}C NMR: 40.59, 44.08, 46.94, 49.95, 67.37, 76.33, 77.98, 110.17, 124.92, 146.85, 151.74, 171.38. LC-MS: *m/z* 236 (*M* + 1). Anal. calcd. for $C_{12}H_{17}N_3O_2$: C, 61.26; H, 7.28; N, 17.86. Found: C, 61.45; H, 7.04; N, 17.91%.

Antioxidant Assays

DPPH radical scavenging assay. The DPPH radical scavenging activity of the test compounds (**Va–l**) was evaluated according to the method of [20]. Initially, 1 mL of the samples at concentrations of 2, 4, 6, 8, and 10 μg/mL were mixed with equal quantity of 0.1 mM DPPH in absolute ethanol. The reaction mixture was shaken and allowed to stand for 20 min at room temperature. Absorbance of the resulting solution was measured at 517 nm with the UV-VIS spectrophotometer. Butylated hydroxyl toluene (BHT) was used as a positive control in all the assays.

Ferrous ion chelating assay. Ferrous ion chelating ability was measured according to Gordon's method [21]. Three sets of test tube were taken. One tube was taken as control: $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added to the tube and the volume was made up to 1mLby adding distilled water. For the second tube, EDTA (40 mM), $FeCl_3$ (200 mM), and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1mL by adding distilled water. For the third one, test compounds (**Va–l**) with concentrations

Table 4. K^+/H^+-ATPase inhibition activity of compounds (**Va–l**)

Test samples	Concentration, μg/mL			
	2	5	10	IC_{50}, μg/mL
(**Va**)	32.61	44.62	68.14	8.5
(**Vb**)	112.36	108.24	102.36	Inactive
(**Vc**)	29.36	53.4	67.14	5.2
(**Vd**)	36.24	56.21	75.39	5.5
(**Ve**)	160.34	147.36	125.36	Inactive
(**Vf**)	33.24	54.36	68.94	6.3
(**Vg**)	37.24	59.24	79.24	4.5
(**Vh**)	26.34	47.32	66.34	4.9
(**Vi**)	22.14	53.5	69.14	5.3
(**Vj**)	152.34	135.58	115.36	Inactive
(**Vk**)	28.69	51.36	64.28	5.7
(**Vl**)	39.24	53.27	73.24	6.2
Omeprazole	24.65	51.32	78.25	4.7

2, 4, 6, 8, and 10 μg/mL, $FeCl_3$ (200 mM), and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1mL by adding distilled water. The tubes were incubated for 10 min at 20°C, the absorbance at 700 nm was registered, and ion chelating ability was calculated.

The antioxidant activity of all the compounds was compared with that of BHT. Radical scavenging activity was expressed as percentage activity using the formula [(Control absorbance – sample absorbance)/control absorbance)] × 100.

Inhibition of Vipera russelli Venom PLA_2

PLA_2 activity was measured by n indirect hemolytic method of Boman [22]. Briefly, packed human erythrocytes, egg yolk, and phosphate buffered saline (1 : 1 : 8 v/v) were mixed. One milliliter of this suspension was incubated separately with 60 μg of *V. russelli* venom for 10 min at 37°C. The reaction was stopped by adding 10 mL of ice-cold phosphate buffer saline and centrifuged at 4°C for 10 min at 800 g. The amount of hemoglobin released into the supernatant was measured at 540 nm. The assay was also carried out in the presence of various concentrations (10, 20, 30, 40, 50, and 60 μg/mL) of compounds (**Va–l**). Lysis of erythrocytes by adding 9 ml of distilled water to the control reaction mixture was taken as 100%.

Gastric H^+/K^+-ATPase Activity

Isolation of parietal cells of stomach. The fundic stomach portion of sheep soon after sacrificing was collected and it was rinsed with Krebs Ringer buffer (250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA, and 2 mM Hepes-Tris, pH 7.4). The upper layer was pined with the help of needles on the dissection Table 4. Mucosal scrapings were suspended in ten volumes of Krebs Ringer buffer (pH 7.4) containing 250 mM sucrose and homogenized with 20 strokes of a mortar-driven Teflon pestle homogenizer. The tissues were discarded and the filtrate was subjected to sub cellular fractionation. The pellets thus obtained were dissolved in 2 mL of sucrose-EGTA buffer and was used as enzyme sample [23].

Protein estimation. Protein was measured by Lowry's method [24] using bovine serum albumin as standard (0–75 μg/mL). Eight clean and dry test tubes were taken and aliquots of various concentrations of the synthesized derivatives were made. To the 7th and 8th test tubes, unknown sample (5 μL and 10 μL of the cells isolated from the sheep stomach) for which the protein content had to be estimated was added. In each test tube, the solution was made up to 1 mL by adding distilled water, which was followed by the addition of 5 mL of Lowry's reagent. All the test tubes were incubated at room temperature for 10 min. Then 0.5 mL of Folin–Ciocalteu reagent was added, followed by incubation at room temperature for 30 min. Absorbance of each solution was read at 670 nm against the blank solution. A graph was plotted by taking the concentration of protein on X-axis and optical density on Y-axis. From the standard graph obtained, the unknown concentration of protein in the enzyme sample was calculated and found to contain 21 mg of protein per 8 g of tissue homogenate.

Inorganic phosphorus estimation. Inorganic phosphorus was estimated according to Fiske's method [23]. Aliquots of working standard solution (40 μg/mL) were added into eight clean and dry test tubes in the volume of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL; 2, 5, and 10 μL of the enzyme sample were taken in test tubes 7 and 8, respectively. The volume in the test tube was made up to 8.6 mL using 10% TCA. Ammonium molybdate (1mL) and ANSA reagent (0.4 mL) were added to each test tube. The tubes were allowed

to stand for 8 min at room temperature. The color developed was read at its λ_{max} of 660 nm.

H^+/K^+-ATPase activity. ATPase activity was determined as described in [25]. Basal Mg^{2+}-dependent ATPase activity was measured in 1.0 mL of the reaction medium consisting of 2 mM ATP and 50 mM Tris-HCl buffer (pH 7.5), K^+-stimulated and HCO_3^- stimulated. ATPase activity was defined as the activity in the basal medium. The ATPase reaction was started by the addition of the substrate (ATP), carried out at 37°C for 15 min, and stopped with 1.0 mL ice-cold 20% TCA; inorganic phosphate liberated from ATP was estimated by Fiske–Subbarow method.

All experiments were repeated in triplicates in three independent sets. Percent inhibition was calculated by the comparison of compound treated to various control incubations. The concentration of test compound causing 50% inhibition (IC_{50}, μg/mL) was calculated from the concentration–inhibition response curve.

CONCLUSION

A new series of piperazine analogues bearing quinoline and pyridine moiety were synthesized and evaluated for antioxidant activity and inhibition of PLA_2 and H^+/K^+-ATPase activities. The result of the present investigations indicates the importance of these new compounds as potential antioxidants and PLA_2 and H^+/K^+-ATPase inhibitors. The compounds presented clearly differ in their activities depending on the type of substitution. Phenyl and nitrophenyl in the piperazine ring of compounds (**Vb**), (**Vc**), and (**Vf**) showed high radical scavenging activities against the tested DPPH and ferrous ions; also, compounds (**Va**), (**Vb**), and (**Vd**) with same groups showed promising inhibition against venom PLA_2. Compounds (**Vg**), (**Vh**), and (**Vi**) with methoxy group on phenyl piperazine indicated selectivity for the H^+/K^+-ATPase. It is remarkable that pyridine ring with phenyl and nitrophenyl group of (**Vc**) and (**Vf**) showed potent inhibition against all the assays. On the basis of above observations, we will do further modification to improve the antioxidant and PLA_2 activities of piperazines.

ACKNOWLEDGMENTS

M. Al-Ghorbani is thankful to the University of Thamar, Yemen, for providing financial assistance under the teacher's fellowship. Dr. S.A. Khanum gratefully acknowledges the financial support provided by the Vision Group on Science and Technology, Government of Karnataka, under the scheme CISEE, Department of Information Technology, Biotechnology and Science & Technology, Bangalore, India.

REFERENCES

1. Maxwell, S.R., *Drugs*, 1995, vol. 49, pp. 345–361.
2. Bedard, K. and Krause, K.H., *Physiol. Rev.,* 2007, vol. 87, pp. 245–313.
3. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J., *Int. J. Biochem. Cell Biol.*, 2007, vol 39, pp. 44–87.
4. Adibhatla, R.M. and Hatcher, J.F., *B. M. B. Rep.,* 2008, vol. 41, pp. 560–567.
5. Penston, J.G. and Wormsley, K.G., *Aliment. Pharmacol. Ther.*, 1992, vol. 6, pp. 3–29.
6. Becker, O.M., Dhanoa, D.S., Marantz, Y., Chen, D., Shacham, S., Cheruku, S., Heifetz, A., Mohanty, P., Fichman, M., Sharadendu, A., Nudelman, R., Kauffman, M., and Noiman, S., *J. Med. Chem.*, 2006, vol. 49, pp. 3116–3135.
7. Smits, R.A., Lim, H.D., Hanzer, A., Zuiderveld, O.P., Guaita, E., Adami, M., Coruzzi, G., Leurs, R., and Esch, I.J., *J. Med. Chem.*, 2008, vol. 51, pp. 2457–2467.
8. Rokosz, L.L., Huang, C.Y., Reader, J.C., Stauffer, T.M., Chelsky, D., Sigal, N.H., Ganguly, A.K., and Baldwin, J.J., *Bioorg. Med. Chem. Lett.*, 2005, vol. 15, pp. 5537–5543.
9. Chen, J.J., Lu, M., Jing, Y.K., and Dong, J.H., *Bioorg. Med. Chem.*, 2006, vol. 14, pp. 6539–6547.
10. Shami, P.J., Saavedra, J.E., Bonifant, C.L., Chu, J.X., Udupi, V., Malaviya, S., Carr, B. I., Kar, S., Wang, M. F., Jia, L., Ji, X.H., and Keefer, L.K., *J. Med. Chem.*, 2006, vol. 49, pp. 4356–4366.
11. Gan, L.L., Lu, Y.H., and Zhou, C.H., Chin. J. Biochem. Pharma., 2009, vol. 30, pp.127–131.
12. Foye, W.O., Lemke, T.L., and William, D.A., *Principles of Medicinal Chemistry,* 4th ed., London: Williams and Wilkins, 1995.
13. Mazzoni, O., Esposito, G., Diurno, M.V., Brancaccio, D., Carotenuto, A., Grieco, P., Novellino, E., and Filippelli, W., *Arch. Pharm.*, 2010, vol. 10, pp. 261–269.
14. Bandgar, B.P., Patil, S.A., Gacche, R.N., Korbad, B.L., Hote, B.S., Kinkar, S.N., and Jalde, S.S., *Bioorg. Med. Chem. Lett.*, 2010, vol. 20, pp. 730–733.
15. Sharma, A., Suhas, R., Chandana, K.V., Banu, S.H., and Gowda, D.C., *Bioorg. Med. Chem. Lett.*, 2013, vol. 23, pp. 4096–4098.
16. Ebrahim zadeh, M.A., Nabavi, S.M., Nabavi, S.F., and Eslami, B., *Pharmacology online*, 2009, vol. 1, pp. 1318–1323.
17. Jayanthi, G.P., Kasturi, S., and Gowda, T.V., *Toxicon,* 989, vol. 27, pp. 875–885.
18. Khanum, S.A., Shashikanth S., and Deepak, A.V., *Bioorg. Chem.*, 2004, vol. 32, pp. 211–222.
19. Ahmed, M., Sharma, R., Nagda, D.P., Jat, J.L., and Talesara, G.L., *Arkivoc*, 2006, vol. XI, pp. 66–75.
20. Scherer, R. and Godoy, H.T., *Food Chem.,* 2009, vol. 112, pp. 654–658.
21. Gordon, M.H. and Hudson, B.J., The mechanism of antioxidant action in vitro, in *Food Antioxidants*, London: Elsevier Applied Science, 1990.
22. Boman, H.G. and Kaletta, U., *Biochim. Biophys. Acta*, 1957, vol. 24, pp. 619–631.
23. Fiske, C.H. and Subbarow, Y., *J. Biol. Chem.,* 1925, vol. 66, pp. 375–400.
24. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., *J. Biol. Chem.,* 1951, vol. 193, pp. 265–275.
25. lm, W.B., Sih J.C., Blakeman, D.P., and Mcgrath, J.P., *J. Biol. Chem.*, 1985, vol. 260, pp. 4591–4597.