Natural Product-Mimetic Scaffolds with Privileged Heterocyclic Systems: Design, Synthesis, and Evaluation of Antioxidant Activity of Quinazoquinobenzothiazinones

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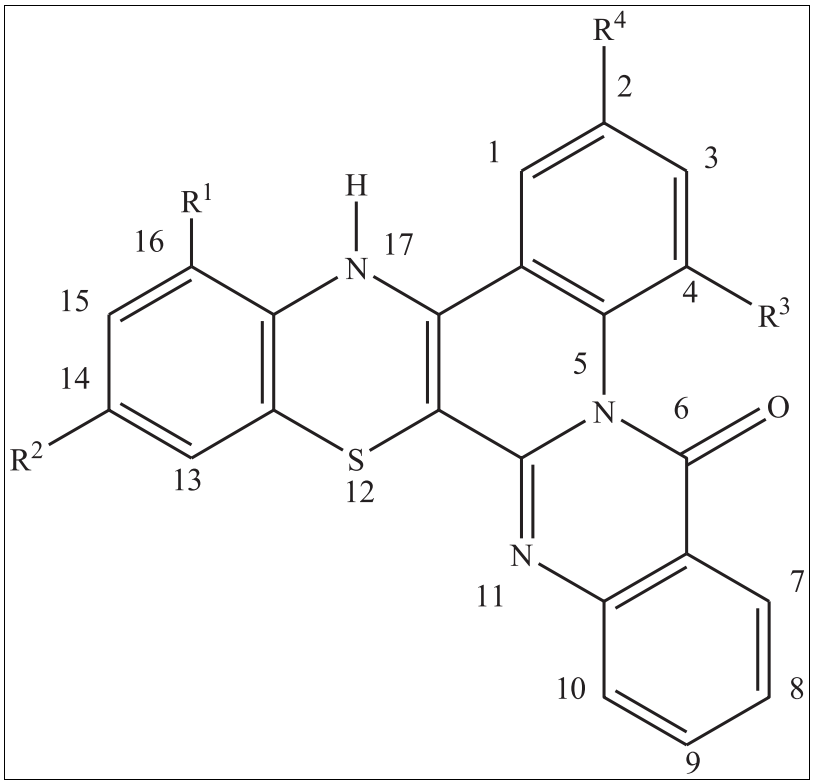
Received November 12, 2014
DOI 10.1002/jhet.2405
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



Structurally diverse quinazolinoquinolinobenzothiazinones based on rutaecarpine structural framework with hybrid structural features of three medicinally privileged heterocyclic systems has been synthesized as natural product-mimetic scaffolds involving the use of multi-step reaction sequences. The synthesized quinazolinoquinolinobenzothiazinones have been evaluated for their antioxidant and radical scavenging activities.

INTRODUCTION

The natural products have been traditionally rich sources of pharmaceuticals, and their role in the drug discovery process has been exceptionally significant. The design and syntheses of natural product-based drug-like small compounds with structural diversity and molecular complexity have been an interesting area of research in modern drug discovery [1,2]. But because of structural complexity due to intricating ring system with large number of stereogenic centers, the syntheses of natural products require very tedious synthetic sequence [3]. To address the problems relating to the synthesis of natural product-based or natural product-like compounds, it is required to identify the basic substructures responsible for the desired biological activity and subsequently simplify the overall structure of the natural product. Rutaecarpine [4] is a major quinazolinocarboline alkaloid present in well-known Chinese herbal drugs, Wu-Chu-Yu [5] and Shih-Hu [6], utilized for the treatment of inflammation-related symptoms [7] in traditional medical practice in China and Japan. Rutaecarpine has been reported to possess a wide spectrum of pharmacological properties, such as calcitonin gene-related peptide (CGRP)-mediated vasodilating, antihypertensive [8], antithrombotic [9], antiplatelet [10], anti-anoxic [11], cardioprotective [12], uterotonic [13], antinociceptive [14], analgesic [15], diuretic [15], specific 2,3,7,8-TCDD

binding inhibitory [16], and cytotoxic activities [17] including selective inhibitory activity against COX-2 isoenzyme.

Quinazolinone is a naturally occurring alkaloid and present as a substructural unit in bioactive natural products and synthetic pharmaceuticals [18]. Quinazolinone is considered to be the privileged structure as it has been extensively utilized as a drug-like scaffold in medicinal chemistry. Quinazolinone natural products luotonine F [19], luotonine A [20], fumiquinazoline [21], mackinazolinone [22], and isoindigotone [22] have shown promising cytotoxic activity (Fig. 1).

Pelanserine [23] is a well-established antihypertensive agent with quinazolinone structural system and comparable in activity with clinically used ketanserin [24]. The interest in quinazolinones as anticancer agents further increased with the discovery of raltitrexed and thymitaq as both the compounds have been proved to be thymidylate inhibitors [25] (Fig. 2).

1,4-Benzothiazines also constitute an interesting class of privileged heterocycles with promising bioactivities [26]. Oxicams containing benzothiazine ring systems are used as non-steroidal anti-inflammatory drugs [27]. Piroxicam is widely used in the treatment of rheumatoid arthritis by blocking the formation of prostagladin through the non-selective inhibition of COX-1/2 isoenzymes [28].

Similarly, quinoline ring system is also a privileged heterosystem and appears in several natural and synthetic pharmaceuticals [29,30]. Xanthosimuline with pyranoquinolone structural motif is active against multidrug resistant KB-VI cancer cells, while huajiasimuline exhibits a selective cytotoxicity profile showing greatest activity with estrogen-positive ZR-75-I breast cancer cells [31].

The annulations or incorporation of two or more privileged heterocyclic structures (pharmacophoric structures) in a single molecule results in structural diversity of the resulting molecule and enhances the biocidal profile remarkably. Therefore, the annulations of heterocyclic-privileged medicinal structures have been one of the best approaches in drug design in generating new structurally diverse natural product-based or natural product-like drugs as the structural diversity is obviously directly related to the compounds potentiality endowed with pharmacological activities. Encouraged by the promising biological activities of the structurally diverse natural product-templated libraries [32] and in continuation of our research program on the synthesis of therapeutically interesting heterocycles [33–35], there has been a need to develop novel synthetic methods, or make systematic use of the known synthetic methods that are more efficient for the synthesis of natural product-based or natural product-like structurally diverse hybrid structures incorporating structural elements of natural products of desired activities. Therefore, in the present work, we have designed and synthesized quinazoquinobenzothiazinones natural product-mimetics

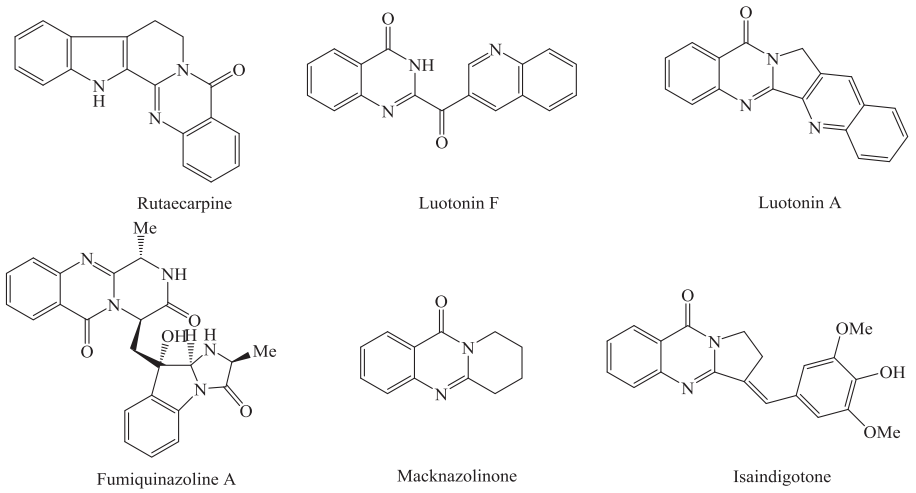


Figure 1. Bioactive quinazolinone natural products.

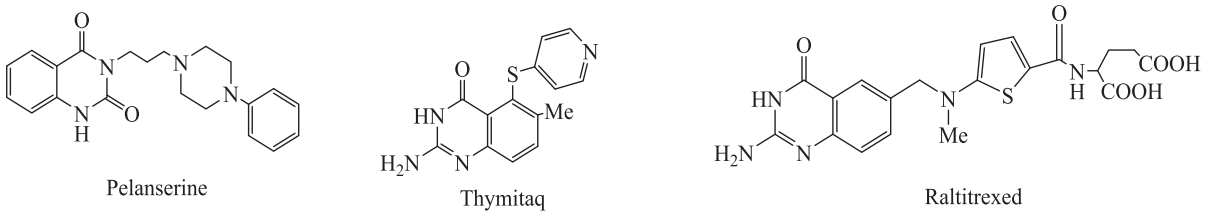


Figure 2. Bioactive quinazolinones derivatives.

based on rutaecarpine structural framework and with hybrid structural features of three privileged biodynamic heterocyclic structures: quinazolinone, quinoline, and benzothiazine, in view to incorporate molecular diversity and structural complexity in the resulting drug-like small molecules, which will provide the promising lead structures for multi-target drug development. The heterocyclic compounds reported in the paper are new and, to our best knowledge, the fused heterocyclic compounds with such three biodynamic-fused heterosystems have not been documented in the literature. The synthesized quinazoquinobenzothiazinones have been evaluated for their antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical cation decolorization assays. The synthesized compounds have also been evaluated for their antioxidant activity in Swiss albino mice. These studies may reflect the possibility for therapeutic uses and as a source of synthetic antioxidants.

RESULTS AND DISCUSSION

Chemistry. 2-Aminobenzenethiols were synthesized by alkaline hydrolysis of 2-aminobenzothiazoles, which, in turn, were prepared by the cyclization of phenylthioureas obtained by the reaction of substituted anilines with ammonium thiocyanate [33a]. 4-Hydroxyquinolin-2-ones were synthesized by the reaction of substituted aniline with malonic ester in the presence of polyphosphoric acid [36]. Quino[3,4-*b*][1,4]benzothiazin-6-(5*H*)-ones (3) have been synthesized in quantitative yields by the reaction of 2-aminobenzenethiols with 4-hydroxyquinolin-2-ones in the presence of dimethyl sulphoxide (DMSO) [34].

Quino[3,4-*b*][1,4]benzothiazin-6-(5*H*)-ones were converted into quinazoquinobenzothiazinones (4) by reacting quino[3,4-*b*][1,4]benzothiazin-6-(5*H*)-ones with phosphoryl chloride in 1,2-dichloroethane at room temperature followed by successive addition of triethylamine and anthranilic acid at 0–5°C. The reaction is considered to proceed via imidoyl chloride formation followed by *in situ* condensation with anthranilic acid and spontaneous cyclodehydration (Scheme 1). The synthesized quinobenzothiazines and quinazoquinobenzothiazinones are included in Table 1.

Biological evaluation. In the present work, a series of structurally diverse new heterocyclic compounds, quinazoquinobenzothiazinones **4a–g**, were synthesized and evaluated for their antioxidant activity by the DPPH radical scavenging and ($ABTS^{+\cdot}$) radical cation decolorizaton assays.

The results of DPPH and $ABTS^{+\cdot}$ inhibition by compounds **4a–g** are summarized in Table 2 and Figure 4. In the present investigation, the compounds **4d**, **4e**, and **4f** showed excellent percent inhibition of $DPPH^{\cdot}$ activity (46.08 ± 1.2%, 45.60 ± 0.09%, and 47.33 ± 0.07%, respectively) and were the most effective DPPH radical scavengers. The percentages can be considered as a full-absorbance inhibition of $DPPH^{\cdot}$, as after completing the reaction, the final solution always possessed some yellowish color, and therefore, its absorbance inhibition compared with the colorless methanol solution could not reach 100% [37,38].

Scheme 1. Reaction sequence for the synthesis of quinazoquinobenzothiazinones.

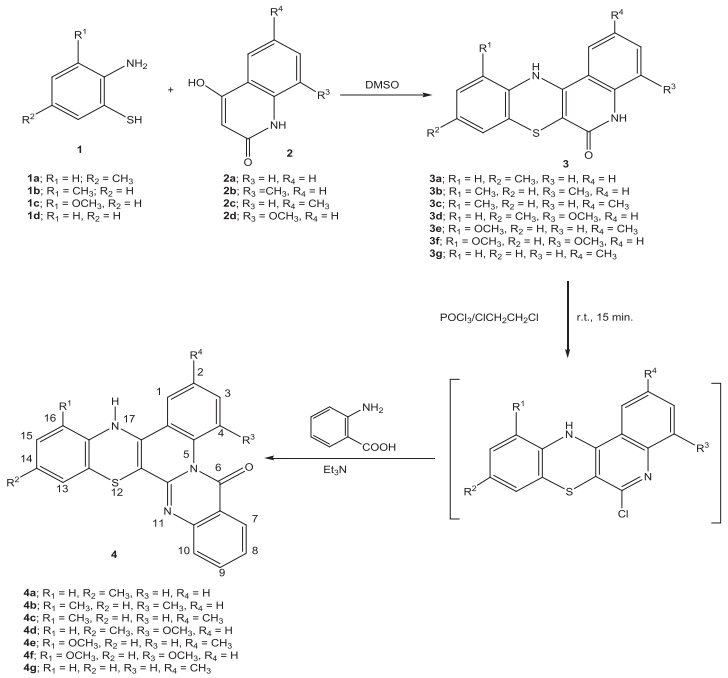
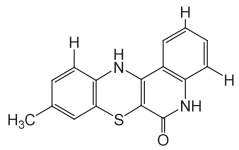
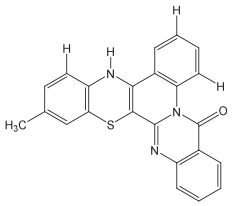
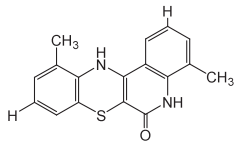
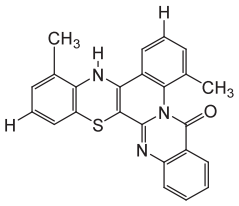
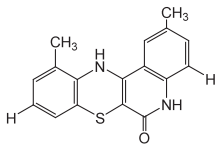
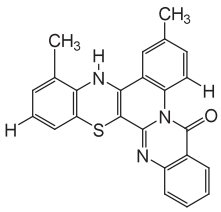
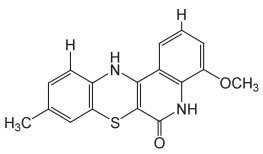
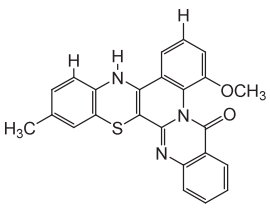
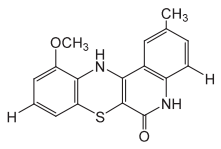
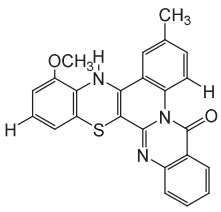


Table 1

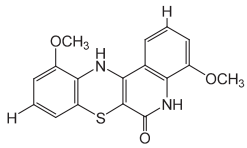
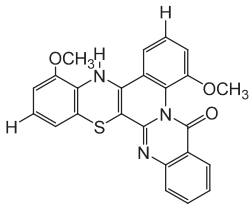
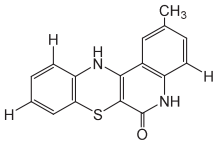
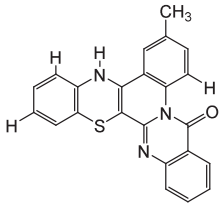
Synthesized quinobenzothiazinones and quinazoquinobenzothiazinones

Quinobenzothiazinones	Quinazoquinobenzothiazinones	Yield (%)
 (3a)	 (4a)	64
 (3b)	 (4b)	68
 (3c)	 (4c)	65
 (3d)	 (4d)	72
 (3e)	 (4e)	70

(*Continued*)

Another antioxidant activity screening method, that is, ABTS radical cation decolorization assay, showed results similar to those obtained by DPPH· assay. The compounds **4d**, **4e**, and **4f** were the most active as they nearly fully scavenged ($ABTS^{+}$). The absorbance after 6 min were 0.296, 0.280, and 0.050, respectively. It was observed that the reaction with ($ABTS^{+}$) was fast in almost all the cases and completed within 1 min.

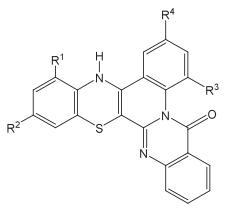
Table 1
(*Continued*)

Quinobenzothiazinones	Quinazoquinobenzothiazinones	Yield (%)
 (3f)	 (4f)	74
 (3g)	 (4g)	62

During the remainder of the reaction time, the changes in absorbance were negligible. Thus, compounds **4d**, **4e**, and **4f** showed the highest radical scavenging activities (RSAs) (Fig. 3).

A significant increase in liver-reduced glutathione (GSH) contents along with decrease in lipid peroxidation (LPO) level was observed in animals treated with compounds **4a–g** (Table 3). However, treatment with compounds **4a**, **4b**, and **4c** showed highly significant increase in GSH contents ($p < 0.005$). Treatment with compounds **4d**, **4e**, and **4f** showed comparatively less significant increase ($p < 0.05$) in GSH contents over the normal (Fig. 4). Also, significant decrease ($p < 0.05$) in LPO level was observed in animals treated with the synthesized compounds (Fig. 5).

Table 2
Antioxidant activity of synthesized quinazoquinobenzothiazinones ($DPPH^{\cdot}$ and ($ABTS^{+\cdot}$) assays).



		($ABTS^{+\cdot}$) activity at different time intervals (min)				
Compounds	$DPPH^{\cdot}$ % inhibition of 1 mg/mL of the compound	0	1	2	4	6
4a	26.72 ± 0.05	0.728	0.510	0.420	0.420	0.420
4b	38.24 ± 0.03	0.732	0.488	0.466	0.466	0.466
4c	27.04 ± 0.15	0.730	0.428	0.310	0.310	0.310
4d	46.08 ± 1.2	0.728	0.320	0.296	0.296	0.296
4e	45.60 ± 0.09	0.722	0.288	0.280	0.280	0.280
4f	47.33 ± 0.07	0.727	0.052	0.050	0.050	0.050
4g	13.47	0.725	0.520	0.510	0.510	0.510

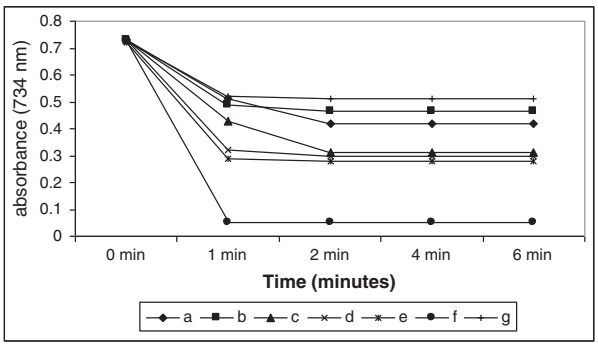
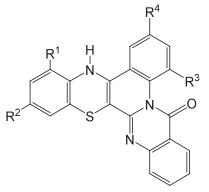


Figure 3. The ABTS activity at different time intervals by quinazoquinobenzothiazinones.

Table 3

Antioxidant properties of quinazoquinobenzothiazinones in liver in Swiss albino mice (LPO and GSH).



Compounds	LPO (nmol/mg tissue)	GSH (nmol/mg tissue)
4a	6.64 ± 0.9 $p < 0.05$	4.83 ± 0.09 $p < 0.005$
4b	6.67 ± 0.11 $p < 0.5$	4.79 ± 0.11 $p < 0.005$
4c	6.58 ± 0.07 $p < 0.05$	4.78 ± 0.07 $p < 0.005$
4d	6.78 ± 0.13	4.62 ± 0.17 $p < 0.05$
4e	6.76 ± 0.05 $p < 0.5$	4.54 ± 0.14
4f	6.79 ± 0.18 $p < 0.5$	4.58 ± 0.16 $p < 0.05$
4g	6.63 ± 0.16 $p < 0.05$	4.80 ± 0.22 $p < 0.005$

LPO, lipid peroxidation; GSH, glutathione.

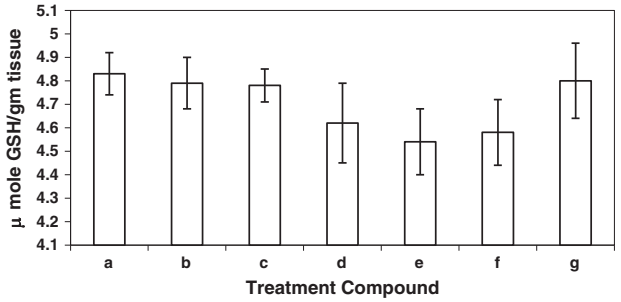


Figure 4. Antioxidant influence of quinazoquinobenzothiazinones on GSH level in liver of Swiss albino mice. GSH, glutathione

CONCLUSIONS

In conclusion, we have designed and synthesized quinazoquinobenzothiazinones based on the natural product, rutaecarpine, structural framework incorporating hybrid structural features of three medicinally privileged

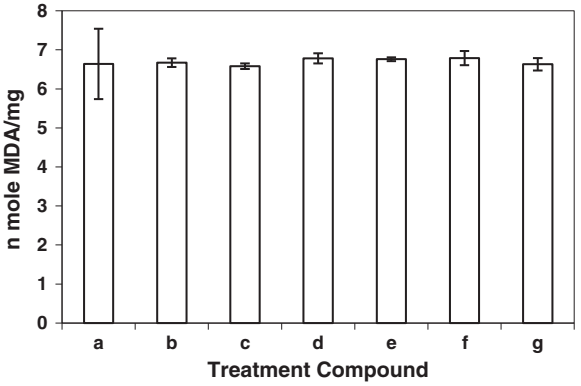


Figure 5. Antioxidant influence of quinazoquinobenzothiazinones on LPO content in liver of Swiss albino mice. LPO, lipid peroxidation

heterocyclic structures: quinozolinone, quinoline, and benzothiazine. The synthesized compounds have been evaluated for their antioxidant activity by $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ assays. The synthesized compounds **4d**, **4e**, and **4f** have shown significant antioxidant activity as interpreted by the results of $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ assays. The synthesized compounds have also shown interesting antioxidant activity as measured by estimating reduced GSH and LPO in the livers of Swiss albino mice. The antioxidant activities of these compounds are attributed to the presence of quinazoquinobenzothiazine heterosystem (hexacyclic-fused heterocyclic system with N–H bonds). Along with, we observed that the presence of substituents such as OCH_3 and CH_3 on the aromatic ring also play an important role in deciding antioxidant activity. The efficiency of this method enabled us to generate rutaecarpine-based natural product-mimetic scaffolds. In addition, the designed and synthesized compounds with such privileged structures will be used as promising medicinal lead structures in the development of multi-target drugs as the medicinally privileged heterocyclic substructures will be capable of binding multiple biological targets (receptors) through interactions.

EXPERIMENTAL SECTION

General method. The melting points of all the synthesized compounds were determined on electric melting point apparatus and are uncorrected. All organic starting materials are analytically pure and used without further purification. The purity of all the synthesized compounds was checked by TLC. The IR spectra were recorded on Shimadzu 8400S FTIR spectrometer (Tokyo, Japan). ^1H NMR and ^{13}C NMR were recorded on JEOL AL NMR spectrometer at 300 and 75 MHz (Tokyo, Japan), respectively. Analytical and spectral data (ir, ^1H NMR, ^{13}C NMR, and ms) of the synthesized heterocycles are also included.

Typical procedure for the synthesis of quinobenzothiazinones (3a–g). Quinobenzothiazinones have been synthesized by a simple and convenient method involving heterocyclization of 2-aminobenzenethiols with 4-hydroxyquinolin-

2-ones in the presence of DMSO. In this method, substituted 2-aminobenzenethiol (0.01 mol, **1a**, **1b** = 1.390 g, **1c** = 1.550 g, **1d** = 1.250 g) was added to the stirred suspension of 4-hydroxyquinolin-2-one (0.01 mol, 1.61 g) in DMSO (6 mL). The resulting mixture was refluxed for 1 h. The mixture was cooled down to room temperature, and the product separated was filtered and crystallized from methanol.

Typical procedure for the synthesis of quinazoquinobenzothiazinones (4a–g). To a mixture of the appropriate quino[3,4-*b*][1,4]benzothiazin-6-(5*H*)-one (0.01 mol, **3a** = 2.8034 g, **3b** = 2.943 g, **3c** = 2.943 g, **3d** = 3.103 g, **3e** = 3.103 g, **3f** = 3.260 g, **3g** = 2.800 g) in 1,2-dichloroethane (10 mL), phosphorylchloride (0.015 mol, 2.299 g) is added, and the mixture is stirred at room temperature for 15 min. Triethylamine (0.015 mol, 1.517 g) is added with stirring at 0–5°C, followed by drop-wise addition of a solution of anthranilic acid (0.01 mol, 1.371 g) in 1,2-dichloroethane (2.5 mL) maintaining the temperature at 5–10°C. The mixture is stirred at room temperature for 1 h and then refluxed for 1 h on a steam bath. Cold water (10 mL) is added to the reaction mixture, the organic layer is separated, washed with 10% aq. Na_2CO_3, water, dried (Na_2SO_4), and concentrated to give 4. The products are purified by recrystallization from EtOH.

Pharmacology – activity evaluation 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay. The DPPH RSA evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the RSA of the specific compounds [39]. A freshly prepared solution of DPPH exhibits deep purple color with an absorption maximum at 517 nm. When an antioxidant is added to the solution of DPPH, its purple coloration disappears because of the quenching of DPPH-free radical and converted into colorless product 2,2-diphenylpicrylhydrazine with the decrease in absorbance. Therefore, the antioxidant activity depends on the absorbance, more rapidly the absorbance decreases, more potent will be the compound for scavenging free radicals.

The RSAs of the compounds **4a–f** were determined against stable DPPH radical spectrophotometer. According to this method, a stock solution (1 mg/mL) of the compound was prepared in methanol, and 50 µL of the compounds were added to 5 mL of a 0.004% methanol solution of DPPH radical with vigorous shaking. The solution was then incubated in the dark at room temperature for 30 min and the absorbance was observed against a blank at 517 nm. The assay was carried out in triplicate, and the RSAs were expressed as inhibition percentage and calculated using the following formula:

Radical scavenging activity

$$\% \text{ Inhibition} = \frac{(\text{AB-AA})}{\text{AB}} \times 100$$

where AB = absorbance of the control (blank, without compound) and AA = absorbance of the compound.

2,2-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolorization assay. The ($ABTS^{+}\cdot$) decolorization test [40] was also used to evaluate the antioxidant activity of compounds **4a–f**. ($ABTS^{+}\cdot$) was generated by oxidation of ABTS with potassium persulphate. For this purpose, ABTS was dissolved in deionized water to 7 m*M* concentration, and potassium persulphate was added to a concentration of 2.45 m*M*. The reaction mixture was left at room temperature overnight (12–16 h) in the dark before its use. The ($ABTS^{+}\cdot$) solution was then diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After the addition of 1 mL of the diluted ($ABTS^{+}\cdot$) solution (A 734 nm = 0.700 ± 0.020) to 10 µL of the compound, the absorbance readings were taken at 30°C exactly 1 min after initial mixing and up to 6 min. All determinations were carried out in triplicate.

***In vivo* studies in Swiss albino mice.** The compounds were further treated for evaluation of antioxidative properties in Swiss albino mice. Results showed that there was significant decrease in LPO level and elevation in reduced GSH in Swiss albino mice.

Material and methods. *Animals.* Swiss albino mice were obtained from Jawaharlal Nehru University, New Delhi, India. Random-bred, male Swiss albino mice weighing 24 ± 2 g were used for experiments. These animals were maintained in the animal house at temperature of 24°C ± 3°C.

The mice were divided in two groups. Group-I animals were fed orally with 0.1 mL of double-distilled water only once a day for 7 days before radiation and served as the control group, while animals of Group-II received compound in 0.1 mL of double-distilled water in a similar fashion.

Animals were sacrificed by cervical dislocation, and the liver was perfused *in situ* immediately with cold 0.9% NaCl, and thereafter, carefully removed and rinsed in chilled 0.15 M tris KCl buffer (Texas, USA) (pH 7.4) to yield a 10% (w/v) homogenate. Aliquots (0.5 mL) of this homogenate were used for assaying reduced GSH and LPO.

Chemicals. Synthesized quinazoquinobenzothiazinones, 1,2-dichloroethane, anthranilic acid, DPPH, ABTS, potassium persulphate, trichloroacetic acid (TCA), 5-dithiobis-2-nitrobenzoic acid, thiobarbituric acid, and so forth.

Biochemical studies. *Lipid peroxidation.* The LPO level in the liver was estimated spectrophotometrically by thiobarbituric acid-reactive substances method of Ohkhawa [41] and expressed in terms of malondialdehyde formed per mg protein. In brief, 0.4 mL of microsomal sample was mixed with 1.6 mL of 0.15 M tris KCl buffer to which 0.5 mL of 30% TCA was added. Then 0.5 mL of 52 m*M* thiobarbituric acid was added, and the mixture placed in a water bath for 25 min at 80°C, cooled in ice and centrifuged at room temperature for 10 min at 3000 rpm. The absorbance of the clear supernatant was measured against a reference blank of distilled water at 531.8 nm.

Reduced glutathione. The reduced GSH level was determined by the method described by Moron [42]. Homogenates were immediately precipitated with 0.1 mL 25%. TCA and the precipitate was removed after centrifugation. Free-SH groups were assayed in a total 3 mL volume by the addition of 2 mL of 0.6 m*M* 5-dithiobis-2-nitrobenzoic acid and 0.9 mL 0.2 m*M* sodium phosphate buffer (pH 8.0) to 0.1 mL of supernatant and the absorbance was observed at 412 nm using a UV–VIS systronics spectrophotometer (India). GSH was used as a standard to calculate µmol GSH/g tissue.

Statistical analysis. Results of the biochemical studies were evaluated using student's *t* test.

Compounds names and spectral detail *9-Methyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6(5H)*-one *(3a).* Yield: 63%, mp 279–281°C; ir (potassium bromide): 3440, 3350, 2915, 1690, 1628, 1520, 830 cm^{-1}; ^1H NMR (dimethyl sulfoxide d_6) δ: 2.38 (s, 3H, CH_3), 6.75–7.50 (m, 7H, Ar–H), 8.34 (s, 1H, NH), 10.20 (s, 1H, CONH). *Anal*. Calcd. (%) for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99. Found: C, 68.41; H, 4.22; N, 9.76.

4,11-Dimethyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6 (5H)-one (3b). Yield: 65%, mp 286–288°C; ir (potassium bromide): 3410, 3330, 2900, 1682, 1624, 1494, 818 cm^{-1}; ^1H

NMR (dimethyl sulfoxide d_6) δ: 2.36 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.90–7.89 (m, 6H, Ar–H), 8.35 (s, NH), 10.80(s, CONH); ^{13}C (dimethyl sulfoxide d_6) δ: 17.4 (CH_3), 18.1(CH_3), 115.9 (NHCO–C=C), 116.5 (ArC), 119.9 (ArC), 121.2 (ArC), 122.1 (ArC), 122.4 (ArC), 122.8 (ArC), 127.5 (ArC), 132.2 (ArC), 133.2 (ArC), 135.0 (NHCO–C=C), 147.5 (ArC), 170.4 (CO); ms (m/z): 294 [M^+]. *Anal*. Calcd. (%) for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.34; H, 4.76; N, 9.50.

2,11-Dimethyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6 (5H)-one(3c). Yield: 68%, mp 284–286°C; ir (potassium bromide): 3410, 3340, 2890, 1685, 1622, 1492, 816 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.31 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.02–7.53 (6H, m, Ar–H), 8.41 (s, 1H, NH), 10.91 (s, 1H, CONH). ^{13}C NMR (DMSO-d6) δ: 17.42 (CH_3), 18.0 (CH_3), 115.9 (NHCO–C=C), 116.5 (ArC), 119.9 (ArC), 121.2 (ArC), 122.1 (ArC), 122.4 (ArC), 122.8 (ArC), 127.5 (ArC), 132.2 (ArC), 133.2 (ArC), 135.0 (NHCO–C=C), 147.5 (ArC), 170.4 (CO). ms (m/z): 294 [M^+]. *Anal*. Calcd. (%) for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.39; H, 4.78; N, 9.54.

4-Methoxy-9-methyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6(5H)-one (3d). Yield: 74%, mp 288–290°C; ir (potassium bromide): 3425, 3330, 2830, 1680, 1620, 1450, 1232, 780 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.41 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.92–7.95 (m, 6H, Ar–H), 8.55 (s, 1H, NH), 11.71 (s, 1H, CONH). *Anal*. Calcd. (%) for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 68.67; H, 4.42; N, 8.89.

11-Methoxy-2-methyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6(5H)-one (3e). Yield: 72%, mp 288–290°C; ir (potassium bromide): 3400, 3340, 2870, 1685, 1615, 1450, 1230, 790 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.52 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.94–7.95 (m, 6H, Ar–H), 8.53 (s, 1H, NH), 10.97 (s, 1H, CONH); ms (m/z): 310 [M^+]. *Anal*. Calcd. (%) for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 35.68; H, 4.43; N, 8.91.

4,11-Dimethoxy-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6(5H)-one (3f). Yield: 70%, mp 292–294°C; ir (potassium bromide): 3390, 3325, 2880,1680, 1610, 1490, 1250, 810 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 3.84 (s, 3H, OCH_3), 4.12 (s, 3H, OCH_3), 6.89–7.91 (m, 6H, Ar–H), 8.53 (s, 1H, NH), 11.73 (s, 1H, CONH); ^{13}C (dimethyl sulfoxide d_6) δ: 54.6 (OCH_3), 55.7 (OCH_3), 110.6 (ArC), 115.8 (NHCO–C=C), 119.9 (ArC), 120.2 (ArC), 121.2 (ArC), 122.3 (ArC), 122.4 (ArC), 127.5 (ArC), 133.2 (ArC), 135.2 (NHCO–C=C), 147.5 (ArC), 156.0 (ArC), 158.2 (ArC), 169.6 (CO); ms (m/z): 326 [M^+]. *Anal*. Calcd. (%) for $C_{17}H_{14}N_2O_3S$: 62.56; H, 4.32; N, 8.58. Found: C, 62.57; H, 4.29; N, 8.60.

2-Methyl-6,12-dihydroquino[3,4-b][1,4]benzothiazin-6(5H)-one (3g). Yield: 60%, mp 278–280°C; ir (potassium bromide): 3445, 3340, 2912, 1685, 1628, 1520, 828 cm^{-1}; ^{1}H NMR (DMSO-d6) δ: 2.50 (s, 3H, CH_3), 6.80–7.54 (m, 7H, Ar–H), 8.30 (s, 1H, NH), 10.92 (s, 1H, CONH). *Anal*. Calcd. for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99. Found: C, 68.46; H, 4.21; N, 9.83.

14-Methyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazin-6-one (4a). Mp 310–312°C; ir (potassium bromide): 3260, 3000, 2880, 1670, 1600, 1550, 750 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.33 (s, 3H, CH_3), 6.58–6.73 (m, 4H, Ar–H), 7.18–7.32 (m, 4H, Ar–H), 7.38–7.49 (m, 3H, Ar–H), 8.51 (s, 1H, NH); ^{13}C (dimethyl sulfoxide d_6) δ: 18.1 (CH_3), 99.4 (S–C=C), 115.9 (ArC), 119.4 (ArC), 120.1 (ArC), 122.8 (ArC), 125.2 (ArC), 126.1 (ArC), 126.5 (ArC), 126.8 (ArC), 127.3 (ArC), 127.5 (ArC), 127.9 (ArC), 128.7 (ArC), 129.4 (ArC), 130.2 (ArC), 132.8 (ArC), 134.1 (ArC), 136.4 (S–C=C), 143.7 (ArC), 147.5 (ArC), 164.4 (C=N), 169.5 (C=O); ms (m/z): 381 [M^+]. *Anal*. Calcd. (%) for $C_{23}H_{15}N_3OS$: C, 72.42; H, 3.96; N, 11.02. Found: C, 72.44; H, 3.93; N, 11.0.

4, 16-Dimethyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazine-6-one (4b). Mp 315–317°C; ir (potassium bromide): 3280, 3010, 2885, 1672, 1605, 1570, 760 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.35 (s, 3H, CH_3), 270 (s, 3H, CH_3), 6.35–6.50 (m, 4H, Ar–H), 6.69–7.12 (m, 3H, Ar–H), 7.35–7.52 (m, 3H, Ar–H), 8.40 (s, 1H, NH); ms (m/z): 395 [M^+]. *Anal*. Calcd. (%) for $C_{24}H_{17}N_3OS$: C, 72.89; H, 4.33; N,10.63. Found: C, 72.44; H, 4.22; N, 10.54.

2,16-Dimethyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazin-6-one (4c). Mp 310–312°C; ir (potassium bromide): 3270, 3050, 2882, 1670, 1608,1560, 765 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.30 (3H, s, CH_3), 2.75 (3H, s, CH_3), 6.38–6.50 (m, 4H, Ar–H), 6.70–7.08 (m, 3H, Ar–H), 7.32–7.43 (m, 3H, Ar–H), 8.55 (1H, s, NH); ^{13}C NMR (dimethyl sulfoxide d_6) δ: 15.6 (CH_3), 18.09 (CH_3), 99.1 (S–C=C), 118.7 (ArC), 119.8 (ArC), 120.6 (ArC), 123.1 (ArC), 124.5 (ArC), 125.2 (ArC), 125.7 (ArC), 126.2 (ArC), 126.6 (ArC), 126.8 (ArC), 127.4 (ArC), 127.7 (ArC), 128.4 (ArC), 128.9 (ArC), 132.7 (ArC), 133.7 (ArC), 134.8 (S–C=C), 146.8 (ArC), 147.5 (ArC), 164.4 (C=N), 169.5 (C=O). *Anal*. Calcd. (%) for $C_{24}H_{17}ON_3S$: C, 72.89; H, 4.33; N, 10.63. Found: C, 72.74; H, 4.32; N, 10.60.

4-Methoxy-14-methyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazine-6-one (4d). Mp 312–314°C; ir (potassium bromide): 3350, 3020, 2920, 1660, 1610, 1580, 790 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.60 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 6.34–6.50 (m, 4H, Ar–H), 6.70–6.90 (m, 3H, Ar–H), 7.25–7.40 (m, 3H, Ar–H), 8.58 (s, 1H, NH); ^{13}C NMR (dimethyl sulfoxide d_6) δ: 18.8 (CH_3), 57.3 (OCH_3), 99.2 (S–C = C), 112.8 (ArC), 114.9 (ArC), 117.7 (ArC), 119.5 (ArC), 120.3 (ArC), 122.4 (ArC), 125.2 (ArC), 126.0 (ArC), 126.5 (ArC), 126.7 (ArC), 127.1 (ArC), 127.7 (ArC), 128.6 (ArC), 128.9 (ArC), 133.7 (ArC), 134.9 (S–C=C), 146.8 (ArC), 147.5 (ArC), 154.5 (ArC), 164.2 (C=N), 169.5 (C=O). *Anal*. Calcd. (%) for $C_{24}H_{17}N_3O_2S$: C, 70.05; H, 4.16; N, 10.21. Found: C, 69.97; H, 4.05; N, 10.13.

16-Methoxy-2-methyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazine-6-one (4e). Mp 317–319°C; ir (potassium bromide): 3310, 3012, 2890, 1680, 1620, 1565, 782 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.64 (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 6.42–6.66 (m, 4H, Ar–H), 6.68–6.85 (m, 3H, Ar–H), 7.30–7.42 (m, 3H, Ar–H), 8.61 (s, 1H, NH); ms (m/z): 411 [M^+]. *Anal*. Calcd. (%) for $C_{24}H_{17}N_3O_2S$: C, 70.05; H, 4.16; N, 10.21. Found: C, 69.02; H, 4.08; N, 10.11.

4,16-Dimethoxy-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazine-6-one (4f). Mp 325–327°C; ir (potassium bromide): 3325, 3015, 2900, 1660, 1625, 1570, 780 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 3.38 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3), 6.35–6.55 (m, 4H, Ar–H), 6.67–6.92 (m, 3H, Ar–H), 7.35–7.60 (m, 3H, Ar–H), 8.72 (s, 1H, NH); ms (m/z): 427 [M^+]. *Anal*. Calcd. (%) for $C_{24}H_{17}N_3O_3S$: C, 67.43; H, 4.01; N, 9.83. Found: C, 67.35; H, 3.94; N, 9.74.

2-Methyl-17H-quinazo[2′,3′:2,1]quino[3,4-b][1,4]benzothiazine-6-one(4g). Mp 306–308°C; ir (potassium bromide): 3240, 3008, 2870, 1670, 1555, 1542, 746 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide d_6) δ: 2.65 (s, 3H, CH_3), 6.36–6.90 (m, 4H, Ar–H), 6.70–7.35 (m, 4H, Ar–H), 7.14–7.7.40 (m, 3H, Ar–H), 8.38 (s, 1H, NH); ms (m/z): 427 [M^+]. *Anal*. Calcd. (%) for $C_{23}H_{15}N_3OS$: C, 72.42; H, 3.96; N, 11.02. Found: C, 72.29; H, 3.94; N, 10.88.

Acknowledgments. The authors are thankful to the Head of the Department of Chemistry, University of Rajasthan, Jaipur for providing necessary laboratory and instrumentation facilities. S. K. and K. S. thank UGC and CSIR, New Delhi for fellowships.

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