Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 110 (2013) 324–332

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

# Synthesis, anticonvulsant, antioxidant and binding interaction of novel *N*-substituted methylquinazoline-2,4(1H,3H)-dione derivatives to bovine serum albumin: A structure–activity relationship study

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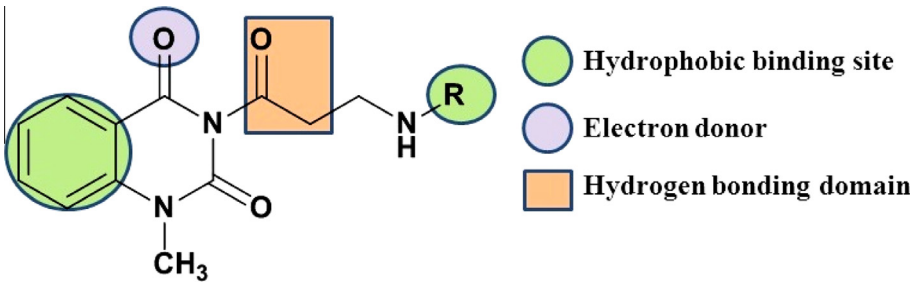
## HIGHLIGHTS

- A new compounds NMQ was synthesized and characterized by $^1$H NMR, IR, and mass spectra.
- Anticonvulsant and antioxidant activity were performed for the prepared compounds.
- We explored the interaction of BSA and NMQ by spectroscopic methods.
- The binding constants, binding sites and binding distance were calculated.
- We have studied the structure–activity relationship to the synthesized compounds.

## GRAPHICAL ABSTRACT

Novel anticonvulsant agents containing essential pharmocophoric elements in their structure.




## ARTICLE INFO

*Article history:*
Received 20 July 2012
Received in revised form 5 November 2012
Accepted 16 March 2013
Available online 25 March 2013

*Keywords:*
Glycosmicine
Anticonvulsant
Antioxidant activity
Bovine serum albumin
Fluorescence spectroscopy

## ABSTRACT

A novel class of *N*-substituted glycosmicine derivatives was synthesized, and their anticonvulsant, antioxidant activity and interaction with bovine serum albumin (BSA) were evaluated. The synthesized compounds **4a–j** were examined for anticonvulsant activity by maximal electroshock induced seizures (MESs) test and their neurotoxic effects were determined by rotorod test in mice. The structure–activity relationships (SARs) of these compounds were also investigated. Compounds **4d**, **4g**, **4i** and **4j** were found to have good protective effect from seizure. The *in vitro* antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging assay. The interaction between novel *N*-substituted methylquinazoline-2,4(1H,3H)-dione (NMQ) and BSA was analyzed by fluorescence and ultraviolet spectroscopy at 304 K under simulative physiological conditions. BSA fluorescence quenched by NMQ is discussed according to the Stern–Volmer equation. The binding constant and binding sites of NMQ with BSA were calculated. According to Forster non-radiation energy transfer theory, the binding distance ($r$) between NMQ and BSA was calculated.



## Introduction

Epilepsy is a heterogeneous mixture of disorders characterized by neuronal hyperexcitability and hypersynchronous neuronal firing, and it affects up to 1% of the world's population [1]. The anticonvulsants are commonly known as antiepileptic drugs, a significant number of effective antiepileptic drugs (AEDs) are now on the market. Classical AEDs comprise phenobarbital, phenytoin, carbamazepine and valproic acid [2]. With the available AEDs on the market, about 70% of people with epilepsy achieve satisfactory seizure control. But these drugs have severe side effects like drowsiness, ataxia, gastrointestinal disturbances, hirsutism and megaloblastic anemia [3–5]. Thus, it is very essential to search for newer chemical entities for the treatment of epilepsy with lower toxicity and fewer side effects.

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http://dx.doi.org/10.1016/j.saa.2013.03.064

In recent years, there has been an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. However, oxygen-derived free radicals resulting from uncontrolled production are hostile and damaging to cells and their functions, and thus play an important role in pathogenesis of some diseases, such as cancer, cardiovascular diseases, atherosclerosis and inflammation [6]. Owing to these facts, there is a constant need for searching new and effective therapeutic agents.

It is known that drug–protein interactions greatly influence the absorption, distribution, metabolism, and excretion properties of drugs [7]. The serum albumin, one of the most abundant proteins, plays an important role in the transport and deposition of a variety of endogenous and exogenous ligands in blood [8]. The most popularly studied albumins are bovine serum albumin (BSA) and human serum albumin (HSA). BSA and HSA display approximately 80% sequence homology [9,10]. BSA has two tryptophan residues that possess intrinsic fluorescence [11]. For its medical importance and ready availability, BSA was usually selected for studying drug–protein interaction *in vitro*.

The quinazolinedione moiety is an important scaffold embedded in a variety of natural alkaloids [12,13]. In recent decades, quinazoline-2,4(1*H*,3*H*)-diones have drawn the attention of chemists and medicinal chemists because of their various biological activities for use as anticonvulsants [14], antibacterial [15], psychosedative [16] and antihypertensive or hypotensive compounds [17]. As such, quinazoline-2,4(1H,3H)-dione derivatives have been widely used as key structures in the production of medicinal drugs [18,19].

Natural products are always special sources for the discovery of potential drugs with novel structures and varying biological activity. The genus *Glycosmis* (Rutaceae) is such an important medicinal plant and has an extensive application in the ayurvedic system of medicine. *Glycosmis arborea* an Indian medicinal plant is used locally against fever, liver complaints and certain other diseases [20]. A wide array of secondary compounds has been isolated from *G. arborea* including carbazole, acridone, furoquinoline, quinoline and quinazoline [21]. They possess a variety of biological effects; *viz* the acridone alkaloids from *G. arborea* including atalaphyllidine, 5-hydroxy-*N*-methylseverifoline, atalaphyllinine, and des-*N*-methylnoracrony-cine showed potent antiproliferative activity against tumor cell lines [22]. Along with acridones, monomeric alkaloids belonging to the carbazole, quinazoline, furoquinoline and quinolone exhibited significant anti-tumor-promoting activity on Epstein-Barr virus early antigen activation [23]. Glycosmicine, one derivative of quinazolinedione has been isolated from the *G. arborea*.

Based on the above literature review and considering the wide applications of quinazolinedione molecule in medicinal chemistry an attempt has been made to synthesize different substituted amine derivatives containing glycosmicine moiety as antiepileptic and antioxidant agents. In addition, the interaction between the NMQ and BSA has been investigated using fluorescence and UV–vis absorption spectroscopy.

## Experimental

### General

All the chemicals and solvents were of AR grade. Solvents were used as supplied by commercial sources without any further purification. BSA (essentially fatty acid free) was purchased from Sigma Aldrich Bangalore, and stored in refrigerator at 4.0 °C. BSA solution was prepared in the Tris–HCl buffer solution (0.05 mol $L^{-1}$ Tris, 0.15 mol $L^{-1}$ NaCl, pH 7.4) and it was kept in the dark at 304 K. The compounds were prepared as a stock solutions using DMF. All other reagents were of analytical reagent grade, and double distilled water was used during the experiment.

### Optical measurements

Elemental analysis (C, H, N) was determined using a Carlo-Erba 1160 elemental analyzer. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using Nujol mulls. The $^1$H NMR spectra were recorded on a Varian AC 400 spectrometer instrument in $CDCl_3$ using TMS as the internal standard. Low resolution mass spectra were obtained on a Varian 1200L model mass spectrometer (solvent: $CH_3OH$). Melting points were determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected. Compound purity was checked by thin layer chromatography (TLC) on precoated silica gel plates (Merck, Kieselgel 60 F254, layer thickness 0.25 mm). The fluorescence measurements were performed on a fluorophotometer (Varioskan Flash 4.00.53) and the UV–vis absorption spectra were recorded with an UV–vis spectrophotometer (Systronics 118, India).

### Synthesis

#### Synthesis of glycosmicine (**2**)

Sodium cyanate (28 mmol) in water (25 mL) was added to a solution of *N*-methyl anthranilic acid (20 mmol) and acetic acid (0.2 mL) in water (50 mL) with stirring. When the temperature of the reaction mixture reached 40 °C, NaOH was added in portions till the reaction temperature reach to 75 °C. Stirring was continued without cooling for 4 h, after which the crystals were filtered off and dissolved in boiling water (50 mL). The solution was acidified with 50% $H_2SO_4$ to pH 1–2. The precipitated crystals were filtered off, washed with water and recrystallized from 50% acetic acid to give compound **2** (2.92 g, 84%); m p 278 °C [24].

#### Synthesis of 3-(3-chloropropanoyl)-1-methylquinazoline-2,4(1H,3H)-dione (**3**)

Compound **2** (6 mmol) was refluxed with 3-chloro propionyl chloride (6.2 mmol) in dry benzene (25 mL) and in the presence of triethylamine (6.2 mmol). Then the reaction mixture was stirred at room temperature for about 8 h. After completion of the reaction (TLC), the reaction mixture was quenched in ice cold water and extracted with dichloromethane. The organic layer was washed with 5% $NaHCO_3$ and dried over $Na_2SO_4$ and concentrated in vacuo to give the light brown product.

Yield: (1.39 g, 87%), m.p.: 257 °C. Anal. calc. for $C_{12}H_{11}ClN_2O_3$: C, 54.05; H, 4.16; N, 10.50. found: C, 54.01; H, 4.15; N, 10.63. $^1$H NMR (300 MHz, $CDCl_3$) δ: 2.73 (t, 2H, CO—$CH_2$), 3.62 (t, 2H, $CH_2$Cl), 3.78 (s, 3H, N—$CH_3$), 7.37–7.88 (m, 4H, Ar—H). IR (Nujol, $cm^{-1}$): 3234.5 (N—H), 3053–2829.5 (Ar C—H), 2971.0–3026.1 ($CH_2$). 1706, 1675, 1635 (C=O).

#### Synthesis of 3-(3-((substitutedphenyl)amino)propanoyl)-1-methylquinazoline-2,4 (1H,3H)-dione derivatives (**4a–j**)

The substituted amines (2 mmol) and triethylamine were taken in THF (20 mL) and anhydrous potassium carbonate was added (600 mg) to it. A solution of **3** (2 mmol), in THF (10 mL) was added drop wise with stirring for 30 min. The reaction mixture was refluxed for 10 h. After completion of the reaction (TLC), the reaction mixture was then desolventized in rotavapor and the compound is extracted in dichloromethane. The dichloromethane layer was washed with water and dried over anhydrous $Na_2SO_4$ and further desolventization in rotary evaporator to get the desired compound. Structure verification data for novel compounds are documented in Supplementary File.

*Pharmacology*

*Animals*

The anticonvulsant activity was evaluated by maximal electroshock seizure (MES) test and neurotoxicity screening. The albino mice (18–20 g) were procured from National Institute of Nutrition, Hyderabad and they were used in this study. The animals were kept in individual cages for 1 week to acclimatize for the laboratory conditions. They were allowed to free access of water and food.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEAs) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee, G Pulla Reddy College of Pharmacy, Hyderabad, India.

*Maximal electroshock seizure model (MES)*

In this study, seizures were induced in mice by delivering electro shock of 150 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. The test compounds (100 mg/kg) were administered by oral route in the form of solution (The compounds were dissolved in 1% sodium carboxymethyl cellulose), 30 min before the maximal electroshock seizure test. The animals were observed closely for 2 min. The percentage of inhibition of seizure relative to control was recorded and calculated [25]. Phenytoin (100 mg/kg, p.o) was used as a standard drug.

*Neurotoxicity screening*

The minimal motor impairment was measured in mice by rotorod test. The mice were trained to stay on the accelerating rotorod that rotates at 10 revolutions per min. Trained animals were given an ip injection of the test compounds at dose of 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least one min in each of the four trials.

*Statistical analysis*

The experimental data were analyzed by one way analysis of variance (ANOVA) followed by Dunnet test to compare the difference between the groups.

*Antioxidant activity*

*DPPH free radical scavenging activity*

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [26]. The test samples (10–100 μL) were mixed with 1 mL of DPPH (0.1 mmol) solution and filled up with methanol to a final volume of 4 mL. Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer (Model 166, Systronics, India). The free radical scavenging rate of the reaction solution was calculated as a percentage (%) of DPPH decoloration using the equation:

$$I(\%) = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

here $A_{\text{blank}}$ is the absorbance of the control reaction mixture excluding the test compounds and $A_{\text{sample}}$ is the absorbance of the test compounds. Radical scavenging potential was expressed as $IC_{50}$ value, which represents the sample concentration at which 50% of the DPPH radicals scavenged. Tests were carried out in triplicate and the results were expressed as mean values ± standard deviations.

*Superoxide scavenging assay*

Superoxide dismutase activity was measured as described by reported method [27]. The assay is based on the reduction of nitroblue tetrazolium (NBT) by superoxide ions generated by the xanthine–xanthine oxidase (X–XO) system. The reaction system contained 0.2 mmol xanthine and 0.6 mmol NBT in 0.1 mol phosphate buffer (pH 7.8). The tested compounds were dissolved in methanol. The reaction was started by addition of XO (0.07 U $mL^{-1}$), an activity which allowed to yield the absorbance change between 0.03 and 0.04 per minute at 560 nm. The extent of NBT reduction was followed spectrophotometrically by measuring the increase of the absorbance at 560 nm. The $IC_{50}$ of each compound was defined as the concentration which inhibited 50% of the NBT reduction by superoxide anion radical produced in the X–XO system.

*Measurement of binding parameters*

For each of the four active compounds (**4a, 4e, 4i** and **4j**), the binding parameters with BSA molecules were measured by fluorescence spectroscopy. A solution (2.5 mL) containing $1.00 \times 10^{-5}$ mol $L^{-1}$ BSA was titrated by successive additions of $1.00 \times 10^{-3}$ mol $L^{-1}$ NMQ stock solution and the concentration of NMQ varied from 0 to $2.40 \times 10^{-5}$ mol $L^{-1}$. Titrations were performed manually using micro-injector. Fluorescence spectra were measured in the range of 280–500 nm at the excitation wavelength of 280 nm. The fluorescence spectra were performed at 304 K.

The UV–vis absorption spectra of NMQ solution at $1.00 \times 10^{-5}$ mol $L^{-1}$ concentration were measured in the range of 200–500 nm at 304 K.

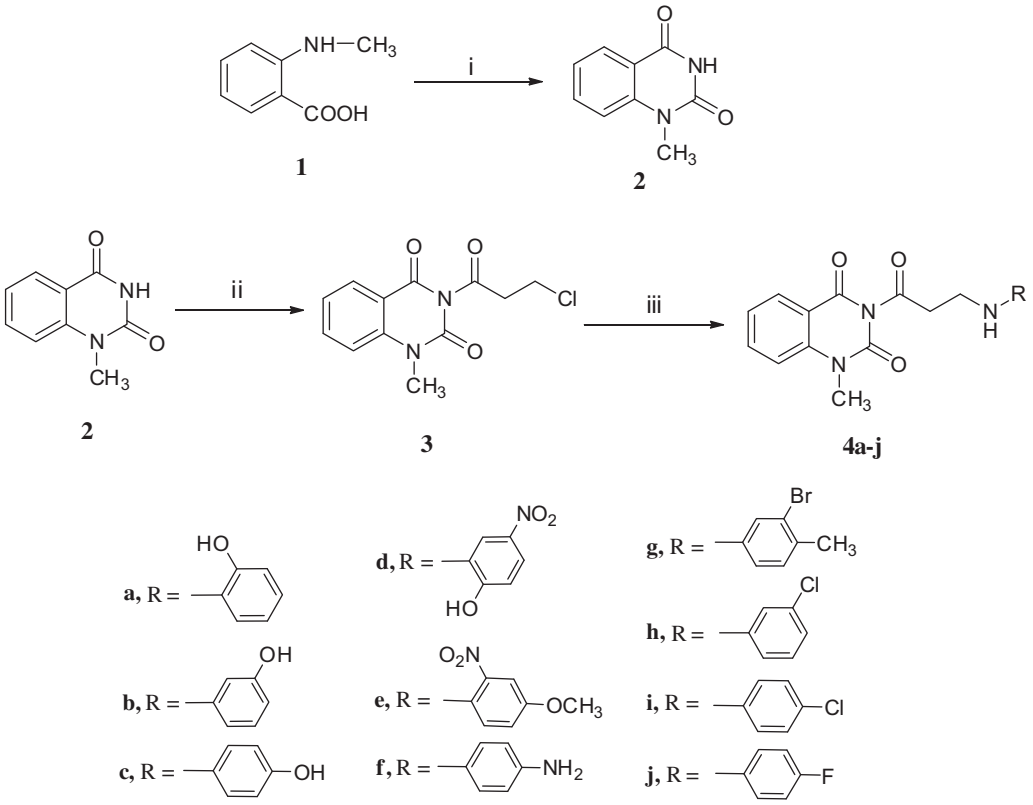
## Results and discussion

*Chemistry*

In view of the above findings, it was considered of interest to undertake the synthesis of *N*-substituted glycosmicine derivatives, hoping that these compounds might possess certain anticonvulsant and antioxidant activity. The syntheses of the compounds **4a–j** were accomplished according to the reaction sequence illustrated in Scheme 1. The structures of the synthesized compounds were deduced on the basis of $^1$H NMR, IR and mass spectra. The composition of all the compounds was obtained by elemental analysis. In addition, the chemical shift and multiplicity patterns correlated well with the proposed structures. The elemental analysis data showed good agreement between the experimentally determined values and the theoretically calculated values.

The naturally occurring plant derived 1-methylquinazoline-2,4(1*H*,3*H*)-dione (glycosmicine) **2** was made by cyclization of *N*-methyl-anthranilic acid **1** with sodium cyanate in basic media. Synthesis of 3-(3-chloropropanoyl)-1-methylquinazoline-2,4(1*H*, 3*H*)-dione **3** as a key intermediate was achieved by the *N*-acylation of glycosmicine **2** with 3-chloro propionyl chloride in presence of triethylamine. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the intermediate, glycosmicine **2** showed resonance at 8.57 ppm (s, 1H, NH), but it was not observed in compound **3**. The two characteristic peaks at 2.73 and 3.62 ppm due to —$CH_2$ were observed in compound **3**. IR spectrum revealed that the presence of three carbonyl groups at 1706, 1675 and 1635 $cm^{-1}$. This confirmed the *N*-acylation of glycosmicine **2**.

Finally, 3-(3-chloropropanoyl)-1-methylquinazoline-2,4(1H,3H)-dione **3** was allowed to base condensation on treatment with different substituted amines in THF and afforded the compounds **4a–j** in good yields (see Supporting Information File 1 for full experimental




**Scheme 1.** Reagents and conditions: (i) sodium cyanate, NaOH, 4 h; (ii) 3-Chloro propionyl chloride, TEA, benzene, 8 h; (iii) substituted amine (a–j), THF, reflux, 10 h.

data). TLC was run throughout the experiment to optimize the reaction for purity and completion. The IR spectra of compounds **4a–c** showed broad phenolic stretching at 3220–3500 $cm^{-1}$ and the appearance of strong absorption band at 3300–3380 $cm^{-1}$ is due to the stretching vibration of N—H bond formation in the synthesized compounds. The $^1H$ NMR spectra of compounds **4a–j** showed singlet at δ 5.62–8.86 which revealed the presence of N–H proton and singlet at δ 3.71–3.83 due to N—$CH_3$ group. The signal due to phenolic OH in all the analogues appeared as singlet at δ 9.02–9.66. The $^1H$ NMR spectrum of compound **4g** showed singlet of —$CH_3$ at 1.48 ppm. Compound **4f** showed singlet at 5.72 ppm due to —$NH_2$ group. The aromatic cluster of compound also supports the synthesis of compounds. Mass spectra of all newly synthesized compounds showed $M+1$ peak, in agreement with their molecular formula.

*Pharmacology*

The new derivatives obtained from the reaction sequence were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES) and neurotoxicity screens, using doses of 100 mg/kg at four different time intervals (0.5, 1, 2 and 4 h). The pre-clinical discovery and development of new chemical agents for the treatment of epilepsy are based mainly on the use of predictable animal models, from which the MES seizure model remain the "gold standards" in early stages of discovery of new AEDs and have gained an appreciable degree of predictability [28,29]. The MES test is associated with the electrical induction of the seizure. The neurotoxicity of the compounds was primarily determined in the rotorod method [30]. The characteristic feature of this series is the presence of the propionyl group at third position and the different substituents on phenyl ring.

Compounds **4b, 4c, 4d, 4e, 4g, 4h, 4i** and **4j** exhibited anticonvulsant activity against MES-induced seizure at the dose of 100 mg/kg (Table 1). It was the indicative of the good ability of these compounds to prevent the seizure spread. The most active of these compounds were **4d, 4g, 4i** and **4j** which showed 63.82–71.98% protection and among these **4i** and **4j** were recorded 70% of protection. The compound **4h** accessible MES effect by 50%, while compounds **4b, 4c** and **4e** revealed 30–42% protection. In the neurotoxicity screening, the compounds were administrated by oral route at 100 mg/kg in mice. The tested compounds did not showed neurotoxicity at 0.5 and 1 h. The results showed compounds having 2-hydroxy **4a**, 2-nitro 4-methoxy **4e** and 4-amine **4f** substituents showed 25% neurotoxicity compared to the standard at 2 h of oral administration (Table 2).

*Antioxidant activity*

The *in vitro* antioxidant activity for compounds **4a–j** was determined by the use of the DPPH test and the results are given in Table 3. The stable free radical DPPH is a useful reagent to investigate the scavenger properties of phenols, catechols and anilines. This assay evaluates the ability of the substance under examination to donate hydrogen atoms or electron donating (conceivable) and quench the visible absorption at 517 nm of the nitrogen radical of DPPH [31]. There are many reports describing the antiradical effect of phenolic compounds by DPPH assay [32,33].

Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easily oxidizable substrates. It can be seen, from Table 3, compounds **4a, 4b, 4c, 4e** and **4f** present the highest scavenging activity on DPPH radical, whereas the **4d** and **4g** present moderate and **4h, 4i** and **4j** present very low scavenging activity on DPPH radical. The results showed significantly higher activity for compounds **4c** and **4f**. The activity of **4c** and **4f** is remarkably higher than that of naturally occurring glycosmicine **2** (21.7 ± 0.38 μg/mL) and other compounds, the main reason might be that the free hydroxyl or amine group is at the position 4 [34,35]. The wide variations in free radical scavenging

**Table 1**
Anticonvulsant activity of the newly synthesized compounds in the maximal electroshock seizure test.

| Treatment | $E/F$[a] | % Protection |
|---|---|---|
| 4a | 5.17 | 04.08 |
| 4b | 3.76 | 30.24[b] |
| 4c | 3.11 | 42.30[b] |
| 4d | 1.95 | 63.82[c] |
| 4e | 3.19 | 40.80[b] |
| 4f | 5.01 | 07.05 |
| 4g | 1.69 | 68.64[c] |
| 4h | 2.48 | 53.98[b] |
| 4i | 1.58 | 70.68[c] |
| 4j | 1.51 | 71.98[c] |
| Glycosmicine | 2.73 | 49.35[b] |
| Standard | 1.27 | 76.43 |
| Control | 5.39 | – |

Values are expressed as mean ± SE. $n = 6$ animals in each group.
[a] Extension/flexion.
[b] $p < 0.05$.
[c] $p < 0.01$ when compared to control.

**Table 2**
Neurotoxicity screening of the compounds.

| Treatment | Neurotoxicity screen | |
|---|---|---|
| | 2 h | 4 h |
| 4a | 1/4 | 1/4 |
| 4b | 0/4 | 1/4 |
| 4c | 0/4 | 0/4 |
| 4d | 0/4 | 0/4 |
| 4e | 1/4 | 1/4 |
| 4f | 1/4 | 1/4 |
| 4g | 0/4 | 0/4 |
| 4h | 0/4 | 1/4 |
| 4i | 0/4 | 0/4 |
| 4j | 0/4 | 0/4 |
| Glycosmicine | 0/4 | 1/4 |
| Standard | 0/4 | 0/4 |

**Table 3**
Antioxidant activity of the synthesized compounds.

| Compounds | $IC_{50}$ (μg/mL)[a] | |
|---|---|---|
| | DPPH | Superoxide |
| 4a | 18.4 ± 0.17 | 13.8 ± 0.19 |
| 4b | 19.6 ± 0.24 | 17.1 ± 0.16 |
| 4c | 13.9 ± 0.04 | 10.8 ± 0.13 |
| 4d | 23.9 ± 0.12 | 27.7 ± 0.22 |
| 4e | 17.9 ± 0.31 | 23.7 ± 0.14 |
| 4f | 16.4 ± 0.37 | 17.3 ± 0.22 |
| 4g | 27.5 ± 0.27 | 30.4 ± 0.10 |
| 4h | 41.8 ± 0.04 | 43.8 ± 0.19 |
| 4i | 43.2 ± 0.26 | 55.3 ± 0.17 |
| 4j | 47.2 ± 0.16 | 59.0 ± 0.09 |
| 2 | 21.7 ± 0.38 | 23.5 ± 0.19 |
| AA[b] | 12.6 ± 0.43 | Nt[d] |
| BHA[c] | Nt[d] | 13.4 ± 0.29 |

[a] Average of three determinations.
[b] Ascorbic acid.
[c] Butylated hydroxyanisole.
[d] Not tested.

activities may be due to the variations in the proton–electron transfer by the compounds due to difference in their structures and stability [36]. An insight to the structure–activity relationship gives an idea that activity generally increases with number and strength of electron donating groups.

Superoxide effects are involved in many pathological processes, such as inflammation, cancer and aging [37]. The enzymatic superoxide anion radical was generated by the xanthine/xanthine oxidase reaction system [38]. The production of superoxide was estimated by the nitroblue tetrazolium method. Table 1 shows the $IC_{50}$ values of individual compounds by superoxide radical scavenging assay ranging from 10.8 ± 0.13 to 59.0 ± 0.09 μM. According to the results, the relative $IC_{50}$ of **4c** was about five times higher than that of **4j** and about two times higher than glycosmicine **2** (23.5 ± 0.19 μg/mL). Compound **4c** has hydroxyl group at para position, so it showed relatively higher superoxide radical scavenging than other phenolics examined.

*Structure–activity relationship*

The most common structural elements of the older generation clinically active anticonvulsants can be defined as a nitrogen hetero-atomic system bearing one or two phenyl rings and at least one carbonyl group. The hypothesis suggests the presence of an aryl hydrophobic binding site, hydrogen bonding domain, an electron donor group and another hydrophobic–hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant. In the present series of compounds, the active compounds possess all the requirements essential for anticonvulsant activity as proposed by Dimmock and others [39]. Thus, our new proposed pharmacophore model includes all the above factors important for bioactivity (Supplementary Fig. 1).

Quinazolinones are a class of drugs which function as sedative-hypnotics; the sedative-hypnotic (neurotoxic) properties of quinazolinones are well documented [40]. Initial structure–activity relationship can be drawn for the synthesized analogues. The results of bioevaluation led to an understanding of the structure–activity relationship of these compounds. All the compounds **4a–j** exhibited promising anticonvulsant activity. SAR studies indicated that different substitution on the aromatic ring, exerted varied anticonvulsant activity.

It was observed that compound **4a** has hydroxyl group at ortho position and **4f** has amine group at para position as substituents showed least activity while compounds (**4i** and **4j**) substituted with 4-chloro and 4-fluoro at para position of the phenyl ring exhibited the maximum percent protection against seizures induced by MES. Compounds (**4b** and **4c**) having electron donating hydroxyl group at 3rd and 4th position of phenyl ring causes decreased in activity. On the other hand, replacement of the fluoro moiety in **4h**, with amine in **4f**, chloro in **4h** and nitro moiety in **4d** and **4e** substitutions reduced the biological activity. This emphasizes that the hydrophobic and lipophilic domains in the molecule are responsible for the potent anticonvulsant activity. Interestingly, the simple phenyl ring with fluoro substitution in para-position exhibited the most potent activity and did not exhibit neurotoxicity at highest administered dose. It may be proved that substitution of a small lipophilic group like fluorine at the para-position of the phenyl ring of this type of compounds resulted in increased activity. From the SAR studies, it reveals that, the substitution of electron donating or electron withdrawing groups to phenylaminopropanoyl at *N*-terminal in glycosmicine ring plays key role in the anticonvulsant activity.

The SAR studies indicate that the electron donating hydroxyl and methoxy goups in **4d** and methoxy group in **4f** are more likely to have higher antioxidant activity. The presence of hydroxyl groups on the phenyl ring greatly influenced antioxidant activity as observed for all of the compounds of this study. By introducing different substituents in various positions of the aromatic ring, it was shown that 4-hydroxy, 4-amine and 2-nitro-4-methoxy substituted compounds were the most active compounds. Modifi-

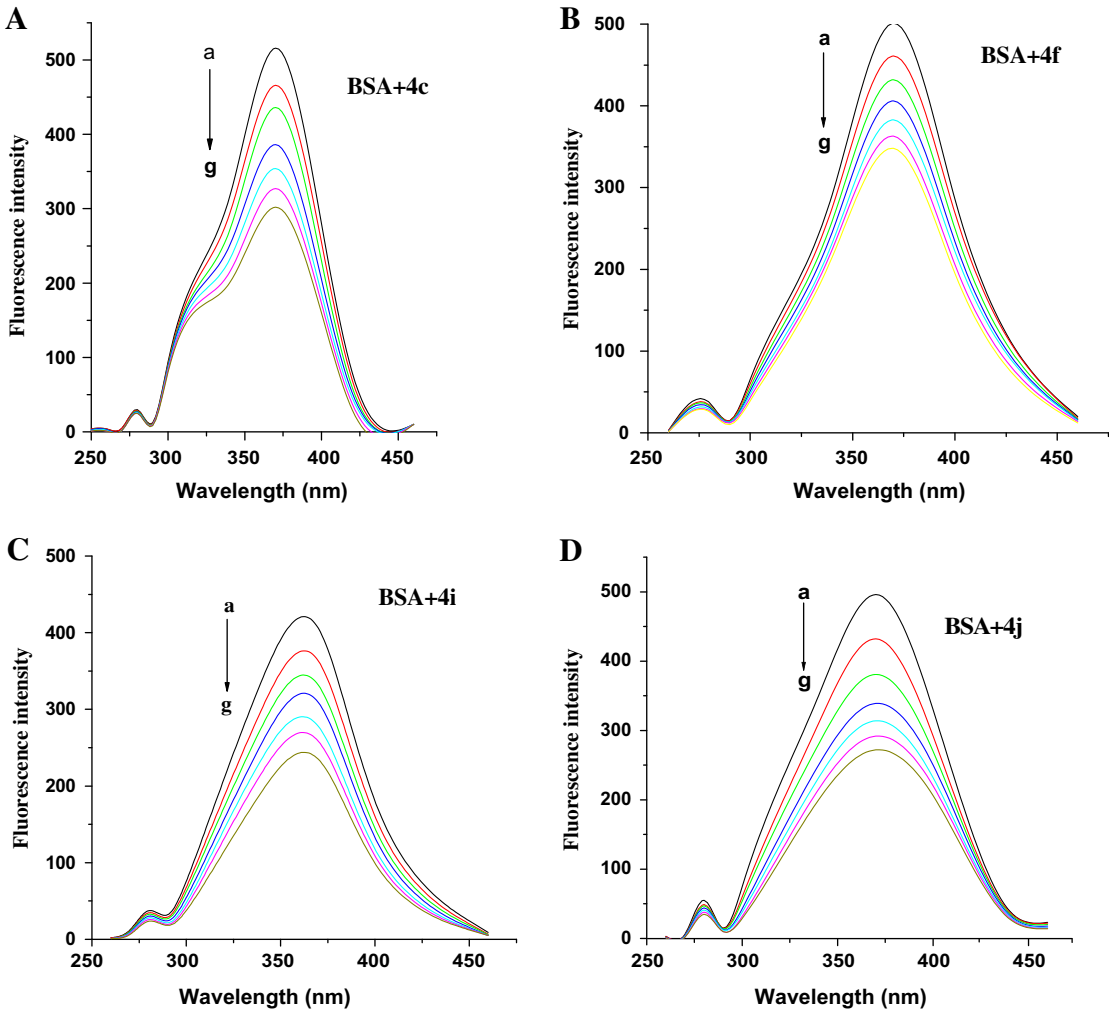



**Fig. 1.** Fluorescence quenching spectra of BSA at different concentrations of NMQ. c(BSA) = $1.00 \times 10^{-5}$ mol $L^{-1}$ ; c(NMQ)/($\times 10^{-5}$ mol $L^{-1}$) (4c, 4f, 4i, 4j): 0, 0.40, 0.80, 1.20, 1.60, 2.00, 2.40 from a to g respectively.

cation of the relative position of the hydroxy moiety from para to ortho and meta resulted in a clear decrease in antioxidant activity (**4c**, **4a** and **4b**). The difference in radical scavenging activity of the **4a, 4b** and **4c** were due to the difference in the stability of the oxygen centered radical formed in these compounds. As in the case of compound **4a**, the radical is stabilized by an intramolecular hydrogen bond. Additionally, there is a considerable difference between **4b** and **4c**, probably due to different electronic effects of the OH groups. The better activity of compound **4c** having hydroxyl group at *p*-position in the aromatic ring is due to high electron releasing properties (positive mesomeric effect is higher than negative inductive effect) and this activates the aromatic ring and stabilizing the corresponding radical. Differently, in the *m*-position, the OH group acts as an electron withdrawing group, providing a destabilizing effect [41,42].

As can be deduced from the structure–activity relationship, position of the groups on the molecule affects the electron donating effect and the scavenging potency of the molecules [43]. When the electron donating groups were replaced by an electron withdrawing group like chloro or fluoro group of phenyl ring resulted in lower the activity. For example, the 3-chloro in **4h**, 4-chloro in **4i** and 4-fluoro in **4j** substituent derivatives were less potent than **4a, 4b, 4c** and **4d**. The above SAR results suggest that the nature and position of functional groups are always related to the favorable antioxidant activity.

*The mechanism of fluorescence quenching of BSA by compounds*

The fluorescence quenching of bioactive NMQ derivatives (**4c, 4f, 4i** and **4j**) and BSA are shown in Fig. 1. The fluorescence quenching spectra of solutions containing BSA in fixed concentration were recorded in the presence of increasing amount of NMQ. As shown in Fig. 1, BSA exhibits a characteristic fluorescence spectrum with a maximum at 370 nm with excitation wavelength of 280 nm. The fluorescence intensity of BSA decreased regularly with increasing concentration of NMQ but there is no significant emission wavelength shift which suggested that the NMQ interact with BSA and quenches its intrinsic fluorescence without altering the environment in the vicinity of the chromophore tryptophan residues.

In order to clarify the fluorescence quenching mechanism, the fluorescence quenching data are analyzed by the Stern–Volmer equation [44]. Eq. (1) can be applied to determine $K_{SV}$ by linear regression from the Stern–Volmer plot of $F_0/F$ against [NMQ] (Fig. 2). Where $F_0$ and $F$ are the relative fluorescence intensities of BSA in the absence of and in the presence of quencher, dynamic and static quenching can be distinguished by the quenching constant $K_q$. $K_{SV}$, $\tau_0$ and [Q] are the Stern–Volmer dynamic quenching constants, average life-time without quencher ($\tau_0 = 10^{-8}$ s) [45] and the concentration of the quencher, respectively.

$$F_0/F = 1 + K_{sv}[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

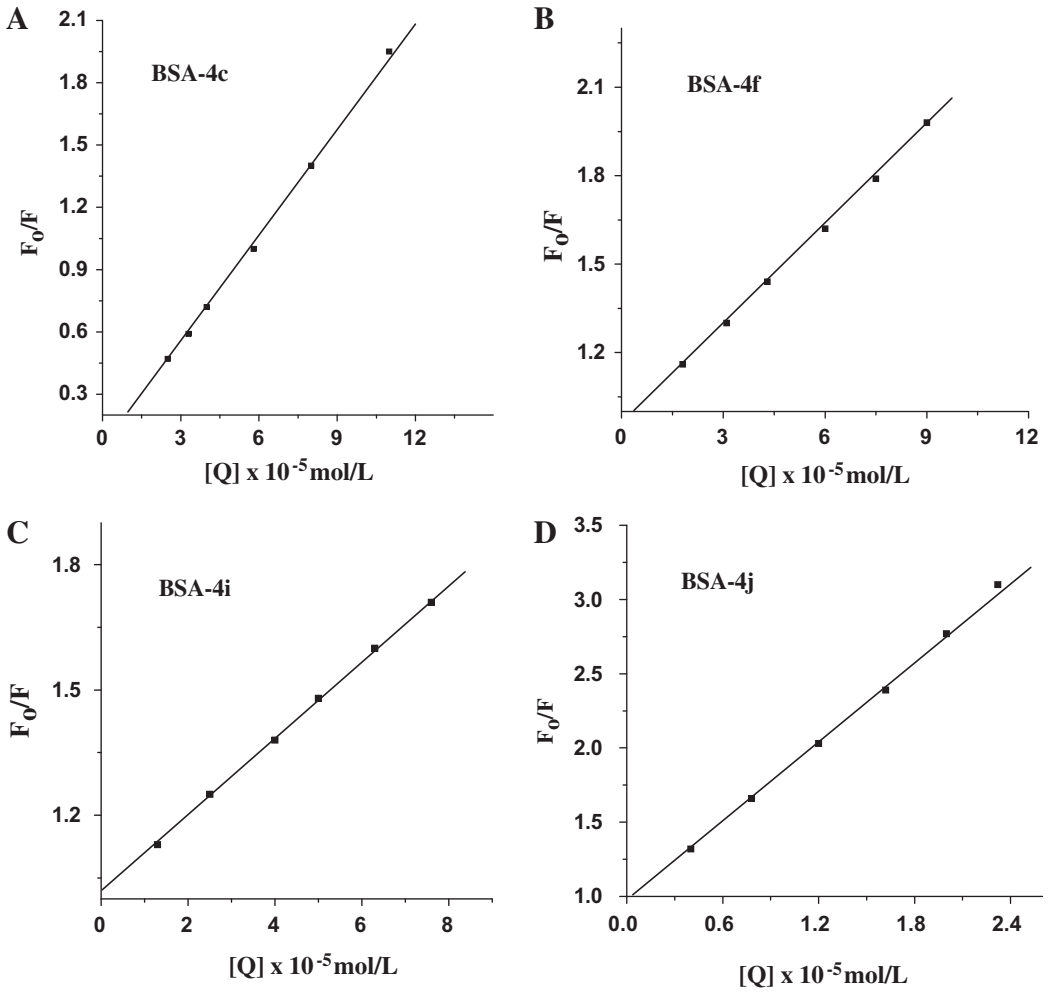



**Fig. 2.** Stern–Volemr plots $F_0/F$ against [NMQ].

**Table 4**
The quenching constants of BSA by compounds.

| Compound | $K_{sv}$ (L mol$^{-1}$) | $K_q$ (L mol$^{-1}$ s$^{-1}$) | $R^a$ |
|---|---|---|---|
| 4c | $2.32 \times 10^4$ | $2.32 \times 10^{12}$ | 0.9973 |
| 4f | $9.43 \times 10^3$ | $9.43 \times 10^{11}$ | 0.9994 |
| 4i | $9.92 \times 10^4$ | $9.92 \times 10^{12}$ | 0.9986 |
| 4j | $7.90 \times 10^4$ | $7.90 \times 10^{12}$ | 0.9972 |

[a] Linear quotient.

**Table 5**
The binding constants and the number of binding sites of compounds with BSA.

| Compound | $K_a$ (L mol$^{-1}$) | $n$ | $R$ |
|---|---|---|---|
| 4c | $3.14 \times 10^4$ | 1.03 | 0.9981 |
| 4f | $1.60 \times 10^4$ | 1.06 | 0.9987 |
| 4i | $5.14 \times 10^5$ | 1.17 | 0.9991 |
| 4j | $1.46 \times 10^5$ | 1.27 | 0.9994 |

**Table 6**
The distance parameters between compounds and BSA.

| Compound | $J$ (cm$^3$ L mol$^{-1}$) | $R_0$ (nm) | $E$ | $R$ (nm) |
|---|---|---|---|---|
| 4c | $4.97 \times 10^{-14}$ | 2.28 | 0.31 | 2.79 |
| 4f | $1.67 \times 10^{-14}$ | 2.68 | 0.07 | 4.12 |
| 4i | $1.06 \times 10^{-14}$ | 2.49 | 0.06 | 3.75 |
| 4j | $2.35 \times 10^{-14}$ | 2.81 | 0.28 | 3.04 |

The Stern–Volmer plots at 310 K are shown in Fig. 2. From Fig. 2, the values of $K_{Sv}$ and $K_q$ were obtained and are listed in Table 4. The observed values of $K_q$ was larger than the maximum scattering collision quenching constant $2.0 \times 10^{10}$ L mol$^{-1}$ s$^{-1}$, which suggest that the fluorescence quenching mechanism between NMQ and BSA may be a static quenching [46].

*Binding constant and binding site*

The binding parameters were then obtained according to the following equation:

$$\log(F_0 - F)/F = \log K_a + n \log[Q] \quad (2)$$

When small molecules bind independently to a set of equivalent sites on a macro-molecule, the equilibrium between free and bound molecules is given by the equation [47]. Fluorescence intensity data can also be used to obtain the binding constant ($K_a$) and the number of binding sites ($n$).

The plot of $\log[(F_0 - F)/F]$ versus log[NMQ] is as shown in Fig. 2 and Table 5 gives the corresponding calculated results. The correlation coefficients ($R$) are larger than 0.995, indicating that assumptions underlying the derivation of Eq. (2) are reasonable, and the curves have good linearity [48]. It showed that the value of $n$ almost equals to 1 indicated that there is one class of binding site for these four NMQ to BSA molecule.

The substitutions on the phenyl ring could enhance the binding affinity of BSA and NMQ. As shown in Table 5, the binding

constants of the interaction between them increased in the following order: **4c < 4f < 4i < 4j**, which indicates that the presence of fluorine atom and chlorine groups strengthen the interaction of NMQ with BSA molecules. The binding affinity of these two compounds may be affected by the polarity of substituent groups. The stronger polarity of substituent group which strengthened its binding affinity. In this work, the molecular size and polarity plays a significant role in the binding between NMQ and BSA.

*Energy transfer from BSA to compound*

The distance between the buried BSA and the interacted NMQ can be estimated by Forster's non-radiative energy transfer theory and the overlapping of fluorescence spectra of BSA with absorption spectra of NMQ are depicted in S Fig. 2 (Supplementary file).

According to Forster's resonance energy transfer theory (FRET), the effective energy transfer from donor to acceptor can happen under the following conditions: (a) the donor can produce fluorescence, (b) fluorescence emission spectrum of the donor and absorption spectrum of the acceptor have enough overlap, and (c) the distance between the donor and the acceptor is shorter than 7 nm.

The efficiency of energy transfer in biochemistry can be used to evaluate the distance between the donor and the fluorophores in the protein [49]. Using FRET, The energy transfer efficiency $E$ and the distance between the acceptor and donor $r$ can be defined as the following equations [50]:

$$E = 1 - (F/F_0) = R_0^6/R_0^6 + r^6 \quad (3)$$

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \Phi J \quad (4)$$

where $r$ is the distance between the acceptor and donor; $F$ and $F_0$ are fluorescence intensity of BSA in the presence and absence of quencher, respectively; $R_0$ is critical energy transfer distance when the transfer efficiency is 50%. $K^2$ is a factor describing the spatial orientation factor related to the geometry of the donor and acceptor of dipoles; $N$ is refractive index of medium; $\Phi$ is fluorescence quantum yield of the donor in the absence of acceptor; $J$ is the effect of spectra overlap between the fluorescence emission spectrum of the donor and the UV–vis absorption spectrum of the acceptor, and $J$ can be calculated by the following equation:

$$J = f(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda / \sum F(\lambda)\Delta\lambda \quad (5)$$

where $F(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range from $\lambda$ to $\lambda + \Delta\lambda$ and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength $\lambda$. In the present case, $K^2 = 2/3$, $N = 1.311$, $\Phi = 0.118$ for BSA molecules [51], respectively. And then, the values of $J$, $E$, $R_0$ and $r$ were calculated and are given in Table 6. The binding distance is shorter than 7 nm which indicates that the energy could transfer from BSA to NMQ [52].

## Conclusion

In this study, we report the synthesis and evaluation of the anticonvulsant activity and neurotoxicity of a series of novel 3-(3-((substituted phenyl)amino)propanoyl)-1-methyl quinazoline-2,4(1*H*,3*H*)-dione derivatives. The syntheses of these compounds have permitted us to further analyze the SAR. However, the most significant result of this work is the finding of a new potent anticonvulsant and antioxidant agents. The compounds **4d, 4g, 4i** and **4j** offer an optimal anticonvulsant efficacy with no neurological toxicity. Compounds **4c, 4e** and **4f** exhibited potent antioxidant activity. The SAR studies reveal that, the substitution nature and position on the phenyl ring affected both anticonvulsant and antioxidant activity remarkably.

The interaction between drug molecule NMQ and BSA was investigated by different spectral methods. The experimental results demonstrated that the intrinsic fluorescence of BSA was quenched *via* dynamic quenching mechanism. The large binding constant values suggest that NMQ binds to the high affinity binding sites of albumins. The biological significance of this work is evident since albumin serves as a carrier molecule for multiple drugs and the interaction of NMQ with albumin was not characterized earlier.

## Acknowledgements

The authors are thankful to the University of Mysore, Mysore for providing this project. The authors are grateful to the MHRD and UGC, New Delhi for the funding through Institution of Excellence Scheme. The authors are grateful to G Pullareddy College of Pharmacy, Mehdipatnam, Hyderabad, India for providing facilities to carry out the anticonvulsant activity. One of the author M. K. Prashanth thankful to the University of Mysore, Mysore for the award of research fellowship.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.03.064.

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