Chapter 4

Synthesis, Characterization and Antioxidant activity of Novel Quinazolinone Functionalized with Urea/Thiourea/Thiazole Derivatives as 5-Lipoxygenase Inhibitors
INTRODUCTION

Inflammation is a multifactorial process. It reflects the response of the organism to various stimuli and is related to many disorders, which all show a high prevalence globally. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals [1]. Reactive Oxygen Species (ROS) lead to diverse pathological conditions, including various liver disorders, atherosclerosis, rheumatic diseases, irradiation and aging by injuring the liver, kidney and other organs [2]. As a result, natural and synthetic small molecules possessing antioxidant activity are becoming increasingly important in disease prevention and therapy.

In addition, ROS propagate inflammation by stimulating release of cytokines and activation of enzymes such as lipoxygenases (LOXs) from inflammatory cells. Lipoxygenases (LOXs) comprise a family of non-heme iron-containing dioxygenases. Lipoxygenases have recently become of interest, as they are considered the key enzymes in the biosynthesis of leukotrienes. Isoenzymes of LOX such as 5, 12 and 15-lipoxygenases have been distinguished based on the peroxidation site of arachidonic acid [3]. These enzymes catalyze the conversion of arachidonic acid to per-oxygenated products at the corresponding sites. The side products of the lipoxygenase reaction are reactive oxygen species (ROS), which determines also the role of lipoxygenase in the pathogenesis of diseases induced by oxidative stress [4]. Among them, 5-lipoxygenase catalyzes the initial step in the metabolism of arachidonic acid leading to leukotrienes and these metabolites are potent physiological effectors in a variety of cellular responses. LOX and their products have been implicated as inflammatory mediators in experimental models of acute lung injury [5] and are known to contribute to the osteoarthritis, asthma and other cancers including prostate, lung, breast and colon [6]. The LOX inhibitors can inhibit the formation of the radical or trap it once formed [7]. For this reason, targeting inhibitors of 5-LOX are a promising therapeutic target for treating wide spectrum of human diseases.

The wide occurrence of the heterocycles in bioactive natural products made them important synthetic targets. In particular, heterocyclic structures form the basis
of many pharmaceutical, agrochemical and veterinary products. One of the most frequently encountered heterocycles in medicinal chemistry is quinazolinone derivatives, for their wide range of pharmacological activities including antioxidant and anti-inflammatory activity [8, 9]. As such, quinazolinone derivatives have been widely used as key structures in the production of medicinal drugs.

**LITERATURE SURVEY**

In the new era of medicinal chemistry the target is focused on the preparation of chemical libraries for the generation of new lead of drug discovery. Heterocyclic chemistry comprises at least half of all research worldwide. Quinazolinones have emerged as an important class of nitrogenated heterocycles. It has been more than a century since the initial studies on 4(3H)-quinazolinones [10] and they are well known as biologically active compounds [11]. Although quinazolinone chemistry is considered to be an established area, newer and more complex variants of the quinazolinone structures are still being discovered. The first reported synthesis of a quinazolinone *i.e.* 2-ethoxy-4(3H)-quinazolinone occurred in 1869 [12]. It was confirmed by the preparation of the derivatives of 2-amino-4(3H)-quinazolinone (1) and 2,4(1H,3H)-quinazolidinedione (2) by reactions with ammonia and water, respectively.

![Chemical Structures]

Majo and Perumal [13] reported the synthesis of 4-(3H)-quinazolinones by dimerization of substituted 2-aminobenzoic acids under Vilsmeier conditions. The interruption of dimerization at room temperature by the addition of primary amines affords the corresponding quinazolinones. In addition, 2-aminobenzoic acid was
Chapter 4  Quinazolinone derivatives

treated with Vilsmeier reagent at 0 °C followed by the addition of a suitably substituted primary amine at room temperature, afforded the corresponding quinazolinones (3, 4).

Larksarp and Alper [14] reported regioselective preparation of 4(3H)-quinazolinone derivatives using palladium-catalyzed cyclocarbonylation of o-iodoanilines with heterocumulenes such as isocyanates, carbodiimides, and ketenimines. A catalyst system comprising palladium acetate-bidentate phosphine is effective for the cyclocarbonylation of o-iodoanilines with heterocumulenes to give the corresponding 4(3H)-quinazolinone derivatives (5-7). The nature of the substrates including the electrophilicity of the carbon center of the carbodiimide and the stability of the ketenimine, which influence the product yields of this reaction. Urea-type intermediates from the reaction of o-iodoanilines with heterocumulenes, followed by palladium-catalyzed carbonylation and cyclization to yield the products.
Barthelemy et al. [15] applied perfluoroalkyl-tagged triphenylphosphine in a fluorous biphasic system for the efficient parallel synthesis of 3H-quinazolin-4-ones (8) via an aza-Wittig reaction. Compounds such as Type 11 were adopted as substrates to react quantitatively with per fluoro-tagged phosphine to obtain iminophosphoranes. Then these intermediates converted directly into the desired quinazoline derivatives through intramolecular aza-Wittig reaction. The reactions were preceded in toluene as solvent and trifluorotoluene as co-solvent. After the reaction, desired products were separated through solid-extraction on fluorous reversed-phase silica gel.

The one-step synthesis of quinazolinones (9) by cyclocondensation of 2-fluoro substituted benzoyl chlorides with 2-amino-\(N\)-heterocycles was reported by Deetz et al. [16]. The reaction proceeds with different combinations of benzoyl chlorides and 2-amino-\(N\)-heterocycles. The products are susceptible to ring-opening attack by alcohols and amines. The compounds were evaluated for anticancer activity and two tetrafluoro quinazolinones were found to be moderately active against a sixty tumor cell lines.

A new solid-phase synthesis of various substituted 2-amino-4(1H)-quinazolinones (10) from a resin bound amine component was described by Gopalsamy and Yang [17]. The amine was readily converted to the corresponding polymer bound \(S\)-methylthiopseudourea. Condensation of resin bound compound with different substituted isatoic anhydrides in a polar aprotic solvent like \(N,N\)
dimethylacetamide led to the formation of the quinazolinone ring. Upon treatment with trifluoroacetic acid afforded 2-amino substituted quinazoline-4-ones.

Bhat and Sahu [18] described the one pot synthesis of 4(3H)-quinazolinones. Anthranilamides undergo cyclocondensation with aldehydes in presence of iodine in a single-pot reaction to afford 2-substituted 4(3H)-quinazolinones (11) under mild conditions. 2,3-Substituted 4(3H)-quinazolinones are synthesized by three-component condensation of isatoic anhydride, amine, and aldehyde in presence of iodine. The synthesized compounds were characterized by NMR, IR, and FAB mass spectra.

Surpur and coworkers [19] have prepared quinazolin-4(1H)-one (12) derivatives by a three-component condensation of isatoic anhydride, a primary amine and an aldehyde catalyzed by amberlyst-15. Reaction was carried out in the presence of different solid Bronsted acid catalysts under microwave irradiation.
Zeghida et al. [20] have reported that, a concise synthesis of 2-amino-4(3H)-quinazolinones from fused heteroaromatic rings from easily accessible (hetero)aromatic amines. The 2-alkylaminoquinazolinone (13) derivatives are obtained in three steps and the key step is the ring closure of the N-protected guanidine intermediates by intramolecular Friedel-Craft’s type substitution.

\[
\begin{align*}
R Н₂ & \xrightarrow{\text{one pot}} \begin{array}{c} \text{CO₂Et} \\ \text{ClSiMe₃} \end{array} \xrightarrow{\text{DMF}} \begin{array}{c} \text{NH} \\ \text{NHR₁} \end{array} \\
& \xrightarrow{\text{DMF}} \begin{array}{c} \text{O} \\ \text{NH} \\ \text{NHR₁} \end{array}
\end{align*}
\]

Li and his group [21] have synthesized the quinazolinones (14) by the condensation of aromatic o-aminonitriles with DMF or N,N-diethylformamide in the presence of ZnCl₂ at 190-200 °C in the sealed reactor. The reaction was not observed when DMF was replaced by N,N-dimethylacetamide.

Zhichkin et al. [22] have demonstrated the synthesis of 2,3-disubstituted 3H-quinazoline-4-one (15) derivatives. It was synthesized by preparation of imidoyl chloride followed by the reaction with a chiral amino acids followed by reductive cyclization. The reduction-cyclization of the resulting chiral N-acyl-2-nitrobenzamide can then be effected under mild conditions avoiding epimerization of the chiral center.

\[
\begin{align*}
& \xrightarrow{\text{S₂Cl₂}} \begin{array}{c} \text{O} \\ \text{R₁} \end{array} \xrightarrow{\text{Boc-L-Ala}} \begin{array}{c} \text{O} \\ \text{R₁} \end{array} \xrightarrow{\text{Zn}} \begin{array}{c} \text{O} \\ \text{R₁} \end{array}
\end{align*}
\]

Xiao-Bi and his coworkers [23] have demonstrated the condensation of anthranilic acid, triethyl orthoformate and amines to synthesize quinazolinones (16) in solvent-free conditions. Different metal perchlorates were screened to catalyze the
three-component reaction and Ni(ClO$_4$)$_2$ or Zn(ClO$_4$)$_2$ was demonstrated to be efficient to catalyze the reaction.

![Chemical structure](image)

Li and Lee [24] carried out the synthesis of quinazoline-2,4-dione derivatives (17, 18) and application to naturally occurring alkaloids from *Zanthoxylum arborescens*. To give the quinazoline-2,4-dione moiety, reaction of isatoic anhydride and tryptamine with triphosgene was next attempted. The synthesis was begins with the reaction of isatoic anhydride with both tryptamine and triphosgene in the presence of K$_2$CO$_3$ in THF. The synthesis of naturally occurring alkaloids was attempted by the reaction of isatoic anhydride with phenethylamine in THF at room temperature for 5 h followed by further reaction with triphosgene in the presence of K$_2$CO$_3$ at room temperature for 15 h afforded 3-phenethylquinazoline-2,4(1H,3H)-dione. This compound was treated with methyl iodide to give the expected products under reflux conditions.

![Synthesis diagram](image)

Sharma and Robert [25] reported the alternative methods to obtain substituted quinazolinones. Preparation of 6-nitro quinazolinone (19) was carried out by readily available starting material 5-nitro-anthranilic acid. The catalysts such as montmorollinate K10, ferric chloride and silica gel were used and microwave conditions are utilized in the reactions.
Very recently, Moghimi et al. [26] have demonstrated the synthesis of a series of 4(3H)- and 4,4’(3H,3H’)-quinoxazoline (20, 21) derivatives and 2-(5-alkyl-1,2,4-oxadiazol-3-yl)quinazolin-4(3H)-one (22). A library of quinoxazolinones was synthesized via the condensation of diaminoglyoxime and anthranilic acid derivatives or methyl 2-amino benzoate and acetic anhydride in acetic acid as the solvent under reflux conditions. The three-component reaction of diaminoglyoxime with two molecules of anthranilic acid was examined under the same reaction conditions to give 2,2’-biquinoxaline-4,4’(3H,3’H)-dione.

In the community of fused heterocycles, 2,3-dihydroquinoxalin-4(1H)-one and 2-spiroquinazolinoine are omnipresent and have been referred to as “core structures” in drug discovery. Recently, Sharma and his group [27] developed a cyanuric chloride catalyzed approach for the synthesis of 2,3-dihydroquinoxalin-4(1H)-one (23), 2-spiroquinazolinoine (24) and glycoconjugates of 2,3-dihydro quinoxalin-4(1H)-one (25) derivatives. The reaction takes place through a cascade comprising (a) mild cyanuric chloride mediated activation of the carbonyl group of
an aldehyde or ketone, which is further attacked by the amine functionality of anthralinamidine to generate the imine and, (b) intramolecular cyclocondensation of imine to furnish the final quinazolinones.

Multicomponent reactions have been successfully adopted by the chemists for the synthesis of a library of biologically active molecules. These reactions have the potential to build complex molecular scaffolds in a straightforward way compared to classical organic synthesis. Recently, Sawant et al. [28] have carried out the synthesis of one-pot multicomponent synthesis of medicinally important quinazolinone derivatives (26, 27). A series of compounds was prepared by cyclization and condensation reactions using microwave. The compounds are structural analogs of anticancer agents IC-87114 and CAL-101, which are isoform-selective PI3K-d inhibitors.

Very recently, Farouk and his group [29] reported the synthetic pathway to synthesize substituted quinazolinediones (28) through the base-catalysed Lossen rearrangement reactions of \( N \)-(sulphonyloxy) phthalimide with amines, hydrazines, and amino acid derivatives. \( N \)-phenylsulphonyloxy-3-nitrophthalimide was
Chapter 4

Quinazolinone derivatives

synthesized as key intermediate compound and its chemical structure was confirmed by $^1$H NMR, IR, and X-ray analysis.

The aforementioned compounds are inspired the idea of synthesizing quinazolinone derivatives where association with a broad spectrum of biologically active molecules, such as thiazole [30], urea [31] and thiourea [32] could be incorporated into the quinazolinone nucleus. In the present study, the author has synthesized quinazolinone nucleus containing urea/thiourea/thiazole derivatives as the antioxidant agents and 5-LOX inhibitors.

EXPERIMENTAL

Materials and methods

All chemicals and solvents were of AR grade. Solvents were used as supplied by commercial sources without any further purification. Elemental analysis (C, H, N) was determined using a Carlo-Erba 1160 elemental analyser. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using Nujol mulls. $^1$H NMR and $^{13}$C NMR spectra were taken on Bruker AV-400 MHz instrument (JEOL LNM-GSX 400a, JEOL JMN-ECP 400,) in DMSO-$d_6$ using TMS as the internal standard. Mass spectra were recorded on Perkin–Elmer LC-MS PE Sciex API/65 spectrometer. 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).

Synthesis of Glycosmicine (12)

A volume of 25 mL aqueous solution of sodium cyanate (28 mmol) 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
to 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% H₂SO₄ to pH 1-2. The precipitated crystals were filtered off, washed with water and recrystallized from 50% acetic acid to give compound 12. Yield: 87%, m.p.: 270 °C.

Anal. calc. for C₉H₈N₂O₂: C, 61.36; H, 4.58; N, 15.90. found: C, 61.33; H, 4.56; N, 15.94. ¹H NMR (400 MHz, DMSO-d₆) δ: 3.76 (s, 3H, N-CH₃), 7.41-7.81 (m, 4H, Ar-H), 8.53 (s, 1H, NH).

IR (nujol, cm⁻¹): 3234, 1706, 1675.

Synthesis of N-(substituted phenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide derivatives (13a-f)

A solution of 1-methylquinazoline-2,4(1H,3H)-dione 12 (0.1 mmol) in dimethylformamide (1 mL) was taken and cooled to 0-5 °C in an ice bath. Triethylamine (0.12 mmol) was added to cold reaction mixture and stirred for 30 min. To the mixture were added different substituted isocyanates (0.1 mmol) and allowed to stir at room temperature for 4 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. The filtrate was concentrated in vacuo to get the crude product which was purified by column chromatography over silica gel (60-120 mesh) using hexane: ethyl acetate (9:1) as an eluent to afford the thiourea in 80-87% yields.

N-(2-Chlorophenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide (13a) Yield: 82%, mp.: 215 °C. Anal. calc. for C₁₆H₁₂ClN₃O₃: C, 58.28, H, 3.67, N, 12.74. found: C, 58.25, H, 3.66, N, 12.77. ¹H NMR (400 MHz, DMSO-d₆) δ: 3.73 (s, 3H, N-CH₃), 7.33-7.75 (m, 8H, Ar-H), 9.96 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 30.2, 115.9, 122.8, 124.6, 124.9, 125.0, 128.1, 129.3, 130.6, 131.5, 133.7, 135.1, 150.2, 150.9, 157.9, 169.5. IR (nujol, cm⁻¹): 3383 (CONH), 1710, 1678, 1635 (C=O), MS, m/z: 330 (M+1), 332 (M+2).

N-(3-Chlorophenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide (13b) Yield: 80%, mp.: 211 °C. Anal. calc. for C₁₆H₁₂ClN₃O₃: C, 58.28, H, 3.67, N, 12.74. found: C, 58.27, H, 3.62, N, 12.81. ¹H NMR (400 MHz, DMSO-d₆) δ: 3.73 (s,
Chapter 4

Quinazolinone derivatives

3H, N-CH$_3$), 7.29-7.73 (m, 8H, Ar-H), 9.91 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ (ppm): 30.4, 115.8, 119.6, 122.7, 123.8, 125.4, 128.0, 128.3, 130.7, 133.5, 134.0, 139.1, 150.4, 151.2, 157.2, 169.8. IR (nujol, cm$^{-1}$): 3380 (CONH), 1701, 1678, 1634 (C=O), MS, m/z: 330 (M+1), 332 (M+2).

$N$-(4-Chlorophenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide (13c) Yield: 84%, mp.: 216 °C. Anal. calc. for C$_{16}$H$_{12}$ClN$_3$O$_3$: C, 58.28, H, 3.67, N, 12.74. found: C, 58.23, H, 3.65, N, 12.83. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 3.72 (s, 3H, N-CH$_3$), 7.31-7.81 (m, 8H, Ar-H), 9.96 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ (ppm): 30.4, 115.6, 120.1, 120.1, 123.6, 124.9, 128.3, 129.3, 129.3, 133.1, 134.4, 138.2, 150.2, 151.5, 157.4, 169.3. IR (nujol, cm$^{-1}$): 3386 (CONH), 1703, 1676, 1632 (C=O), MS, m/z: 330 (M+1), 332 (M+2).

$N$-(4-Fluorophenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide (13d) Yield: 81%, mp.: 223 °C. Anal. calc. for C$_{16}$H$_{12}$FN$_3$O$_3$: C, 61.34, H, 3.86, N, 13.41. found: C, 61.31, H, 3.84, N, 13.47. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 3.70 (s, 3H, N-CH$_3$), 7.25-7.83 (m, 8H, Ar-H), 9.93 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ (ppm): 30.2, 115.6, 120.1, 120.1, 123.6, 124.9, 128.3, 129.3, 129.3, 133.5, 135.9, 150.2, 151.5, 157.1, 164.7, 168.9. IR (nujol, cm$^{-1}$): 3385 (CONH), 1702, 1675, 1630 (C=O), MS, m/z: 314 (M+1).

$N$-(4-Methoxyphenyl)-1-methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carboxamide (13e) Yield: 87%, mp.: 251 °C. Anal. calc. for C$_{17}$H$_{15}$N$_3$O$_4$: C, 62.76, H, 4.65, N, 12.92. found: C, 62.73, H, 4.61, N, 12.97. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 3.71 (s, 3H, N-CH$_3$), 3.81 (s, 3H, OCH$_3$), 7.11-7.89 (m, 8H, Ar-H), 9.97 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ (ppm): 30.2, 54.6, 114.1, 114.1, 115.7, 120.1, 120.1, 124.1, 125.1, 128.3, 133.5, 135.9, 150.2, 151.5, 157.1, 164.7, 169.4. IR (nujol, cm$^{-1}$): 3365 (CONH), 3265 (CONH), 1705, 1677, 1632 (C=O), MS, m/z: 326 (M+1).

1-Methyl-2,4-dioxo-N-(p-tolyl)-1,2-dihydroquinazoline-3(4H)-carboxamide (13f) Yield: 85%, mp.: 237 °C. Anal. calc. for C$_{17}$H$_{15}$N$_3$O$_3$: C, 66.01, H, 4.89, N, 13.58. found: C, 65.98, H, 4.81, N, 13.64. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 2.31 (s, 3H, CH$_3$), 3.70 (s, 3H, N-CH$_3$), 6.93-7.98 (m, 8H, Ar-H), 9.95 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ (ppm): 22.5, 30.4, 115.9, 121.2, 121.2, 123.4, 125.0, 126.2,
129.9, 129.9, 131.4, 134.5, 136.2, 150.2, 151.1, 158.1, 169.8. IR (nujol, cm⁻¹): 3381 (CONH), 1703, 1676, 1632 (C=O), MS, m/z: 310 (M+1).

**Synthesis of 1-Methyl-2,4-dioxo-1,2-dihydroquinazoline-3(4H)-carbothioamide (14)**

Compound 12 (0.1 mmol), hydrochloric acid (9 mL) and water (25 mL) were taken and refluxed for 30 min. The contents were cooled to room temperature and then ammonium thiocyanate (0.1 mmol) was added. The reaction mixture was again refluxed for 4 h. The obtained solid was cooled, filtered, washed with water, dried, and recrystallized from ethanol. Yield: 79%, mp.: 294 °C. Anal. calc. for C₁₀H₉N₃O₂S: C, 51.05, H, 3.86, N, 17.86, S, 13.63. found: C, 51.01, H, 3.80, N, 17.91, S, 13.55. ¹H NMR (400 MHz, DMSO-d₆) δ: 3.71 (s, 3H, N-CH₃), 7.23-7.83 (m, 4H, Ar-H), 8.16 (s, 2H, NH₂). IR (nujol, cm⁻¹): 3320 (NH₂), 1701, 1676 (C=O). MS, m/z: 236 (M+1).

**Synthesis of substituted-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide derivatives (15a-f)**

Compound 14 (0.1 mmol) in dimethylformamide (1 mL) was taken and cooled to 0-5 °C in an ice bath. Triethylamine (0.1 mmol) was added to cold reaction mixture and stirred for 30 min, then different acid chlorides (0.1 mmol) were added, the reaction mixture was allowed to stir at room temperature for 6-8 h. After completion of the reaction (TLC), the reaction mixture was quenched with saturated sodium bicarbonate solution; the product was extracted with ethyl acetate, dried over anhydrous sodium sulfate and evaporation of the solvent affording the corresponding 15a-f.

**3,5-Difluoro-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide (15a)** Yield: 79%, mp.: 177 °C. Anal. calc. for C₁₇H₁₁F₂N₃O₃S: C, 54.40, H, 2.95, N, 11.19, S, 8.44. found: C, 54.38, H, 2.91, N, 11.28, S, 8.36. ¹H NMR (400 MHz, DMSO-d₆) δ: 3.40 (s, 3H, N-CH₃), 7.28-7.91 (m, 7H, Ar-H), 10.6 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 30.8, 109.4, 110.9, 110.9, 114.5, 121.9, 123.8, 125.3, 131.7, 138.1, 151.9, 156.4, 162.3, 163.8, 163.9, 167.4, 177.1. IR (nujol, cm⁻¹): 3357 (N–H), 1708, 1675, 1632 (C=O), MS, m/z: 376 (M⁺1).
Chapter 4  Quinazolinone derivatives

2,3,4-Trifluoro-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonyl)benzamide (15b) Yield: 75%, mp.: 192 °C. Anal. calc. for C_{17}H_{10}F_{3}N_{3}O_{5}S: C, 51.91, H, 2.56, N, 10.68, S, 8.10. found: C, 51.86, H, 2.55, N, 10.66, S, 8.03. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.41 (s, 3H, N-CH\(_3\)), 7.18-7.97 (m, 6H, Ar-H), 10.01 (s, 1H, NH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 30.8, 113.6, 121.3, 122.7, 123.1, 123.9, 125.8, 126.5, 131.6, 141.3, 147.1, 151.9, 154.9, 156.2, 167.5, 169.3, 177.8. IR (nujol, cm\(^{-1}\)): 3345 (N-H), 1706, 1674, 1630 (C=O), MS, m/z: 394 (M+1).

2,5-Difluoro-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide (15c) Yield: 81%, mp.: 172 °C. Anal. calc. for C\(_{17}\)H\(_{11}\)F\(_2\)N\(_3\)O\(_5\)S: C, 54.40, H, 2.95, N, 11.19, S, 8.43. found: C, 54.36, H, 2.91, N, 11.28, S, 8.32. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.69 (s, 3H, N-CH\(_3\)), 7.32-7.88 (m, 7H, Ar-H), 10.16 (s, 1H, NH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 30.8, 112.5, 114.6, 118.1, 120.3, 123.7, 125.9, 126.7, 131.7, 151.2, 155.3, 159.3, 167.9, 169.2, 178.1. IR (nujol, cm\(^{-1}\)): 3358 (N-H), 1701, 1672, 1631 (C=O), MS, m/z: 376 (M+1).

4-Fluoro-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide (15d) Yield: 75%, mp.: 186 °C. Anal. calc. for C\(_{17}\)H\(_{12}\)F\(_{3}\)N\(_3\)O\(_5\)S: C, 57.14, H, 3.38, N, 11.76, S, 8.91. found: C, 57.11, H, 3.35, N, 11.83, S, 8.83. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.82 (s, 3H, N-CH\(_3\)), 7.41-8.03 (m, 8H, Ar-H), 10.01 (s, 1H, NH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 30.8, 113.8, 113.8, 114.2, 121.6, 123.7, 124.8, 127.8, 129.1, 129.1, 136.2, 153.7, 157.1, 164.0, 164.9, 167.8, 176.8. IR (nujol, cm\(^{-1}\)): 3346 (N-H), 1701, 1675, 1630 (C=O), MS, m/z: 358 (M+1).

4-Chloro-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide (15e) Yield: 76%, mp.: 195 °C. Anal. calc. for C\(_{17}\)H\(_{12}\)ClN\(_3\)O\(_5\)S: C, 54.62, H, 3.24, N, 11.24, S, 8.46. found: C, 54.61, H, 3.20, N, 11.27, S, 8.41. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.70 (s, 3H, N-CH\(_3\)), 7.45-7.98 (m, 8H, Ar-H), 10.05 (s, 1H, NH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 30.8, 114.9, 122.1, 123.7, 125.6, 128.1, 130.8, 130.8, 131.1, 132.0, 138.7, 151.7, 155.9, 166.3, 166.5, 168.3, 177.8. IR (nujol, cm\(^{-1}\)): 3360 (N-H), 1701, 1672, 1635 (C=O), MS, m/z: 374 (M+1), 376 (M+2).

4-Hydroxy-N-(1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-3-carbonothioyl)benzamide (15f) Yield: 82%, mp.: 214 °C. Anal. calc. for C\(_{17}\)H\(_{13}\)N\(_3\)O\(_5\)S: C, 57.46, H,
3.69, N, 11.82, S, 8.93. found: C, 57.39, H, 3.65, N, 11.91, S, 8.86. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.42 (s, 3H, N-CH\(_3\)), 7.25-7.99 (m, 8H, Ar-H), 9.57 (s, 1H, OH), 10.05 (s, 1H, NH). \(^13\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 30.7, 115.1, 115.7, 115.7, 122.1, 124.1, 126.2, 127.8, 129.1, 129.1, 132.7, 151.3, 156.4, 161.6, 165.2, 168.1, 176.8. IR (nujol, cm\(^{-1}\)): 3426 (OH), 3361 (N–H), 1703, 1674, 1632 (C=O), MS, m/z: 356 (M+1).

**Synthesis of 1-methyl-3-(substituted thiazol-2-yl)quinazoline-2,4(1H,3H)-dione (16a-d)**

To the compound 14 (0.1 mmol) in DMF were added substituted phenacyl bromides and triethylamine (0.1 mmol) was refluxed for 2 h and then the reaction mixture was poured into water. The reaction mixture was extracted with ethyl acetate and the organic portions were dried over anhydrous Na\(_2\)SO\(_4\). Evaporation of the solvent and recrystallization from ethanol gave the product 16a-d.

1-Methyl-3-(4-phenylthiazol-2-yl)quinazoline-2,4(1H,3H)-dione (16a) Yield: 75%, mp.: 187 °C. Anal. calc. for C\(_{18}\)H\(_{13}\)N\(_3\)O\(_2\)S: C, 64.46, H, 3.91, N, 12.53, S, 9.44. found: C, 64.45, H, 3.86, N, 12.55, S, 9.37. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.65 (s, 3H, N-CH\(_3\)), 7.25-7.91 (m, 10H, Ar-H). \(^13\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 31.2, 106.3, 115.1, 122.9, 124.0, 126.9, 126.9, 128.5, 129.1, 129.7, 129.7, 133.1, 133.6, 150.4, 150.9, 151.3, 159.6, 174.2. IR (nujol, cm\(^{-1}\)): 1703, 1675 (C=O), MS, m/z: 336 (M+1).

1-Methyl-3-(4-(p-tolyl)thiazol-2-yl)quinazoline-2,4(1H,3H)-dione (16b) Yield: 75%, mp.: 210 °C. Anal. calc. for C\(_{19}\)H\(_{15}\)N\(_3\)O\(_2\)S: C, 65.31, H, 4.33, N, 12.03, S, 9.10. found: C, 65.30, H, 4.26, N, 12.05, S, 9.02. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 2.41 (s, 3H, CH\(_3\)), 3.65 (s, 3H, N-CH\(_3\)), 7.23-7.88 (m, 9H, Ar-H). \(^13\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) (ppm): 21.6, 31.2, 32.1, 104.6, 115.1, 123.1, 124.2, 126.5, 126.5, 128.9, 129.7, 129.7, 130.9, 133.0, 150.1, 150.6, 151.3, 159.1, 173.9. IR (nujol, cm\(^{-1}\)): 1701, 1678 (C=O), MS, m/z: 350 (M+1).

3-(4-(4-Methoxyphenyl)thiazol-2-yl)-1-methylquinazoline-2,4(1H,3H)-dione (16c) Yield: 77%, mp.: 219 °C. Anal. calc. for C\(_{19}\)H\(_{15}\)N\(_3\)O\(_3\)S: C, 62.45, H, 4.14, N, 11.50, S, 8.78. found: C, 62.41, H, 4.09, N, 11.52, S, 8.71. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.68 (s, 3H, N-CH\(_3\)), 3.84 (s, 3H, OCH\(_3\)), 7.29-7.92 (m, 9H, Ar-H). \(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\): 30.7, 115.1, 115.7, 115.7, 122.1, 124.1, 126.2, 127.8, 129.1, 129.1, 132.7, 151.3, 156.4, 161.6, 165.2, 168.1, 176.8. IR (nujol, cm\(^{-1}\)): 3426 (OH), 3361 (N–H), 1703, 1674, 1632 (C=O), MS, m/z: 356 (M+1).
Chapter 4  Quinazolinone derivatives

MHz, DMSO-\textit{d}_{6}), \delta (ppm): 31.1, 56.1, 60.9, 104.9, 114.2, 114.2, 115.5, 123.9, 125.1, 125.7, 128.2, 129.6, 129.6, 132.9, 149.7, 150.6, 151.5, 159.7, 174.4. IR (nujol, cm\textsuperscript{-1}): 1703, 1672 (C=O), MS, m/z: 366 (M+1).

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-methylquinazoline-2,4(1H,3H)-dione \textit{(16d)}

Yield: 70\%, mp.: 196 °C. Anal. calc. for C\textsubscript{18}H\textsubscript{12}ClN\textsubscript{3}O\textsubscript{2}S: C, 58.46, H, 3.27, N, 11.36, S, 8.67. found: C, 58.44, H, 3.21, N, 11.38, S, 8.62. \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_{6}) \delta: 3.71 (s, 3H, N-CH\textsubscript{3}), 7.23-7.88 (m, 9H, Ar-H). \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_{6}), \delta (ppm): 31.1, 105.8, 115.1, 123.8, 124.7, 127.9, 128.1, 128.1, 128.9, 128.9, 131.7, 133.0, 135.3, 135.3, 150.6, 151.2, 151.8, 160.3, 174.0. IR (nujol, cm\textsuperscript{-1}): 1702, 1672 (C=O), MS, m/z: 370 (M+1), 372 (M+2).

Antioxidant activity

\textit{The detailed procedure regarding the antioxidant activities of the synthesized compounds (13a-f, 15a-f and 16a-d) using DPPH free radical and superoxide radical scavenging assay is described in Chapter 2.}

Hydroxyl radical scavenging assay

This assay was performed by a method reported by Jayabharathi \textit{et al.}, [33] with a slight modification. Hydroxyl radical scavenging of quinazolinone derivatives was carried out by measuring the competition between 2-deoxyribose and the synthesized compounds for hydroxyl radicals. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with thiobarbutyric acid (TBA). The hydroxyl radicals (·OH) in aqueous media were generated through the Fenton system. The assay was performed by mixing 0.36 mL of 2-deoxyribose (2.8 mM), 0.33 mL of phosphate buffer (20 mM, pH-7.4), 1 mL of test solution (10-100 µL), 0.1 mL of hydrogen peroxide (1 mM), 0.1 mL of ascorbic acid (100 mM), 0.1 mL of EDTA (100 mM) and 0.01 mL of FeCl\textsubscript{3} (100 mM) and this mixture was incubated at 37 °C for 1h. Thereafter, 1mL of cold 2.8% trichloroacetic acid was added and reactivity was developed by adding 1mL of thiobarbutyric acid (1% w/v) followed by heating at 100 °C for 15 min, the absorbance of the cooled mixture was measured at 532 nm (Elico SL-177 visible spectrophotometer, India). As a positive control, butylated hydroxyanisole (BHA) was used. All samples and the control were made in triplicate and the results are expressed as mean values ± standard deviations.
Nitric oxide radical scavenging assay

Nitric oxide radical scavenging capacity is based on the method of Padmaja et al., [34]. The assay is based on generation of nitric oxide (NO) from sodium nitroprusside (SNP) and it was measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be quantified by the Griess Reagent. The reaction mixture containing 1 mL of SNP (10 mM), 1.5 mL of phosphate buffer (pH 7.4) and test solution (10-100 µL) incubated for 150 min at 25 °C. Then, 1 mL of Griess reagent (1 % sulfanilamide in 3 % phosphoric acid and 0.2% \(N\)-(1-naphthyl)ethylenediamine dihydrochloride) was added to the reaction mixture and allowed to stand for 3 min, the absorbance of this solution was measured at 546 nm against the reagent blank. All samples and controls were made in triplicate. IC\(_{50}\) values were determined and the results are expressed as mean values ± standard deviations.

In vitro 5-LOX inhibitory assay

The 5-LOX was assayed by the method described by Frum and Viljoen [35]. Enzyme activity was measured spectrophotometrically as an increase in absorbance at 234 nm. Typical reaction mixture contained various concentrations of test substances in DMSO, 50 µL of linoleic acid and made up to 1 mL with 0.1 M phosphate buffer with Tween. The reaction was initiated with the addition of 1.5 µL 5-lipoxygenase. Percent inhibition was calculated by comparison of LOX activity in the presence and absence of inhibitor. The concentration of the test compound causing 50% inhibition (IC\(_{50}\)) was calculated from the concentration-inhibition response curve. Each assay was repeated thrice.

RESULTS AND DISCUSSION

Synthesis

In an attempt to design and develop novel biologically active compounds, the author has performed the synthesis of some series of potential quinazolinone incorporating urea/thiourea/thiazole derivatives and evaluation of their antioxidant and anti-inflammatory activities. The synthetic strategy for the novel quinazolinone
incorporating urea/thiourea/thiazole derivatives is illustrated in Scheme 1. The structures of the synthesized compounds were deduced on the basis of $^1$H NMR, $^{13}$C NMR, IR and mass spectra. The composition of all the compounds was obtained by elemental analysis. The proton and carbon magnetic resonance spectra of synthesized compounds have been recorded in DMSO-$d_6$. In addition, the chemical shift and multiplicity patterns correlated well with the proposed structures. The chemical structures of all the synthesized compounds are given in Table 1. Compounds gave correct values in elemental analysis.

Scheme 1: Synthesis route of compounds 13a-f, 15a-f and 16a-d. Reagents and conditions: (i) NaOCN, NaOH, 4 h; (ii) R-N=C=O, DMF, TEA; (iii) NH₄SCN, HCl, 4 h; (iv) R-COCl, DMF, TEA, 6-8 h; (v) R-COCH₂Br, DMF, TEA, 2h.

First the key intermediate, 1-methylquinazoline-2,4(1H,3H)-dione (glycosmicine) 12 was prepared by cyclization of N-methyl-anthranilic acid 11 with sodium cyanate in basic media [36]. The syntheses of target amide derivatives (13a-f) were achieved by the coupling methods between the corresponding isocyanates and glycosmicine 12. In the IR spectra of (13a-f), four bands characteristic to these compounds out of which three bands for carbonyl group were observed at the region 1706-1701, 1675-1670 and 1635-1630 cm$^{-1}$ and one band for CONH was found in the range 3386-3365 cm$^{-1}$. The $^1$H NMR spectra of compounds 13a-f revealed a singlet at 9.12-9.41 ppm due to NH proton, singlet at 3.70-3.73 and at 2.31 ppm due to N-methyl and methyl groups of compounds 13a-e and 13f, respectively.
Compound 14 was obtained by the action of ammonium thiocyanate on glucosmicine 12 in acidic medium [37]. IR absorption peak at 3320 cm\(^{-1}\) and 1701, 1676 corresponding to NH\(_2\) and C=O groups, respectively. The proton spectral data of the intermediate, glycosmicine 12 showed resonance at 8.57 ppm (s, 1H, NH), but it was not observed in compound 14. \(^1\)H NMR showing a singlet at 8.16 ppm corresponding to amine group substantiated the formation of thiourea.

The amidation of compound 14 with different substituted acid chlorides in dichloromethane give rise to the respective amide compounds 15a-f. The IR spectrum of compound 15f exhibited a characteristic absorption band at 3426 cm\(^{-1}\) due to phenolic hydroxyl group and absorption bands at 1703, 1674, 1632 cm\(^{-1}\) due to C=O stretching. \(^1\)H NMR of 15a-f showed the signal at 9.13-9.57 singlet (1H, NH) indicating the formation of amide bond between thiourea 14 and acid chlorides and all the aromatic protons exactly matching the structure. The \(^1\)H NMR spectra of 15f exhibited resonances attributable to N-methyl functionality (s, 3H, 3.42), a benzamide functionality (s, 1H, 9.57), and singlet (1H, 11.54), consistent with a hydroxyl functionality.

On the other hand, condensation of compound 14 with phenacyl bromides afforded the corresponding thiazole derivatives 16a-d in excellent isolated yields. A comparative study of spectral data on compounds 14 and 16a-d, revealed that the absence of a signal for amine (at δ 8.16, s, 2H) and a new signal appeared at δ 7.23-7.25 (s, 1H) in the \(^1\)H NMR spectra of the product 16a-d, thus confirming the formation of thiazole compounds. 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.

\textit{NMR and Mass spectra representative compounds are given at the end of this chapter.}

\textbf{Antioxidant activity}

It is well known that rates of reactive oxygen and nitrogen species production are increased in most diseases. Antioxidants play a key role in offering cure to various life-style related diseases. In fact, many non-steroidal anti-inflammatory drugs have been reported to act as radical scavengers. Because different antioxidant
compounds may act through different mechanisms, no single method can fully evaluate the total antioxidant capacity. For this reason, various assays were performed to assess the antioxidant activities of the compounds. Radical scavenging potency of all the compounds 13a-f, 15a-f and 16a-d was assessed in vitro by the DPPH, superoxide, nitric oxide and hydroxyl radical scavenging assay. The results are presented in Table 1.

### Table 1: Antioxidant activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>IC&lt;sub&gt;50 ± SD&lt;/sub&gt; (µg/mL)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DPPH</td>
<td>Superoxide</td>
<td>Nitric oxide</td>
<td>Hydroxyl</td>
</tr>
<tr>
<td>13a</td>
<td>-C₆H₄(o-Cl)</td>
<td>31.9±0.26</td>
<td>30.6±0.41</td>
<td>52.9±0.82</td>
<td>41.5±0.49</td>
</tr>
<tr>
<td>13b</td>
<td>-C₆H₄(m-Cl)</td>
<td>33.6±0.55</td>
<td>32.5±0.15</td>
<td>55.9±0.67</td>
<td>48.1±0.71</td>
</tr>
<tr>
<td>13c</td>
<td>-C₆H₄(p-Cl)</td>
<td>31.5±0.62</td>
<td>31.2±0.33</td>
<td>52.1±0.78</td>
<td>42.9±0.53</td>
</tr>
<tr>
<td>13d</td>
<td>-C₆H₄(p-F)</td>
<td>42.3±0.18</td>
<td>44.7±0.85</td>
<td>58.1±0.51</td>
<td>55.3±0.49</td>
</tr>
<tr>
<td>13e</td>
<td>-C₆H₄(p-OCH₃)</td>
<td>13.1±0.31</td>
<td>10.9±0.14</td>
<td>14.8±0.09</td>
<td>11.7±0.03</td>
</tr>
<tr>
<td>13f</td>
<td>-C₆H₄(p-CH3)</td>
<td>21.3±0.27</td>
<td>18.1±0.15</td>
<td>17.6±0.13</td>
<td>20.7±0.22</td>
</tr>
<tr>
<td>15a</td>
<td>-C₆H₃(3,5-F)</td>
<td>28.1±0.28</td>
<td>28.3±0.72</td>
<td>31.4±0.55</td>
<td>29.6±0.16</td>
</tr>
<tr>
<td>15b</td>
<td>-C₆H₃(2,3,4-F)</td>
<td>30.7±1.02</td>
<td>30.1±0.83</td>
<td>32.7±0.29</td>
<td>31.2±0.56</td>
</tr>
<tr>
<td>15c</td>
<td>-C₆H₃(2,5-F)</td>
<td>30.1±0.23</td>
<td>31.6±0.45</td>
<td>36.2±0.17</td>
<td>34.4±0.71</td>
</tr>
<tr>
<td>15d</td>
<td>-C₆H₄(p-F)</td>
<td>36.4±0.68</td>
<td>42.1±0.73</td>
<td>58.9±0.22</td>
<td>44.7±1.49</td>
</tr>
<tr>
<td>15e</td>
<td>-C₆H₄(p-Cl)</td>
<td>28.3±0.39</td>
<td>27.9±0.82</td>
<td>30.1±0.16</td>
<td>30.2±0.52</td>
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<tr>
<td>15f</td>
<td>-C₆H₄(p-OH)</td>
<td>12.7±0.06</td>
<td>10.2±0.19</td>
<td>13.4±0.64</td>
<td>10.6±0.03</td>
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<tr>
<td>16a</td>
<td>-C₆H₅</td>
<td>26.5±0.27</td>
<td>25.3±0.38</td>
<td>26.1±0.59</td>
<td>26.8±0.24</td>
</tr>
<tr>
<td>16b</td>
<td>-C₆H₄(p-CH3)</td>
<td>23.1±0.13</td>
<td>20.7±0.06</td>
<td>22.9±0.17</td>
<td>23.1±0.54</td>
</tr>
<tr>
<td>16c</td>
<td>-C₆H₄(p-OCH₃)</td>
<td>13.5±0.14</td>
<td>12.6±0.19</td>
<td>14.1±0.53</td>
<td>12.7±0.18</td>
</tr>
<tr>
<td>16d</td>
<td>-C₆H₄(p-Cl)</td>
<td>27.3±0.29</td>
<td>27.1±0.37</td>
<td>28.5±0.11</td>
<td>28.3±0.35</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>21.7±0.38</td>
<td>23.5±0.19</td>
<td>26.3±0.43</td>
<td>31.4±0.19</td>
</tr>
<tr>
<td>AA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>12.6±0.43</td>
<td>n.t&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.t&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.t&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>n.t&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.4±0.29</td>
<td>14.3±0.18</td>
<td>15.3±0.47</td>
</tr>
</tbody>
</table>

<sup>a</sup> ascorbic acid; <sup>b</sup> butylated hydroxyanisole; <sup>c</sup> not tested.

Values are means of triplicate determinations.
The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [38]. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the antioxidant molecule can quench DPPH free radical through donation of hydrogen atom or by electron donating conceivably to form a stable DPPH molecule. Hence, instantaneously or concomitant decrease in absorbance was found, which indicates that the more potent the antioxidant activity of the compound.

Table 1 shows the results obtained for the scavenging effects of all the compounds. It was found that all the newly synthesized compounds exhibited moderate to good activity. An insight to the structure-activity relationship gives an idea that activity generally increases with strength of oxygen containing functional groups. The compounds with substituent such as 4-OMe (13e), 4-OH (15f) and 4-OMe (16c) behaved the stronger DPPH than remain others.

The difference in radical scavenging activity of the 13e, 15f and 16c were due to the difference in the stability of the oxygen centered radical formed in these compounds. It is clear from the results that the antioxidant potential of compounds is associated with the position of the substituents on phenyl ring. Methyl-substituted compounds 13f and 16b have shown moderate activities and chloro-substituted derivatives 13a, 13b, 13c, 15e and 16d have shown least activity compared with the standard, ascorbic acid.

Superoxide anion radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to the tissue damage and various diseases. Superoxide anion is produced by several oxidative enzymes as a product in the one-electron reduction of oxygen [39]. Xanthine oxidase is one of the major oxidative enzymes producing superoxide anion. The enzymatic superoxide anion radical was generated by a xanthine and xanthine oxidase reaction system. It is well known that superoxide radical itself is not a “super” redox agent, but is a key upstream source of highly oxidizing derivatives, such as hydroxyl radicals and reactive nitrogen species [40].
Superoxide radical scavenging activity was measured as described by reported method [41]. The compounds 13a-f, 15a-f and 16a-d were found to be moderate to weak super oxide radical scavenger. The IC\textsubscript{50} values of these compounds were in the range 10.2 to 44.7 µg/mL. The IC\textsubscript{50} value of 13e, 15f and 16c derivatives on superoxide radical scavenging activity was found to be 10.9±0.14, 10.2±0.19 and 12.6±0.16 µg/mL, respectively, which was significantly higher than that of the standard BHA. Compounds 13d and 15d exhibited lesser activity, and their IC\textsubscript{50} values were observed at 44.7 and 42.1 mg/mL, respectively.

Among the oxygen radicals, hydroxyl radicals are the most reactive and they induce severe damage to the adjacent biomolecules [42]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, its significant role as an initiator of lipid peroxidation is well documented. The scavenging effect of hydroxyl radical was investigated using the Fenton reaction and the results are shown in Table 1. When the test compounds were added to the reaction mixture, they removed hydroxyl radicals from the sugar and prevented their degradation. Compounds 13e, 15f and 16c exhibited the remarkable capacity for scavenging hydroxyl radical with IC\textsubscript{50} value of 11.7, 10.6 and 12.7 µg/mL, respectively.

It is well known that nitric oxide has an important role in various inflammatory processes. Despite the beneficial effects, an overproduction of this reactive nitrogen species is associated with several types of biological damage. Apart from its role in physiological processes, 'NO also has toxic properties, especially after reaction with oxygen or superoxide anion radicals. The reaction products which are formed highly reactive peroxynitrite anion are able to inflict severe cellular damage [43]. The 'NO scavenging activity indicates that the OH and OCH\textsubscript{3} substitution in the phenyl group is the main factor responsible for the scavenging effect of this reactive nitrogen species. Compounds 13e, 13f, 15f and 16c were the most effective ones with IC\textsubscript{50} value 14.8, 17.6, 13.4 and 14.1µg/mL, respectively.

The analogue 15f has hydroxyl group at position 4 of its phenyl ring, thus demonstrating its antioxidant effect. Moreover, the methoxyphenolic moiety has also been reported to be an essential structural feature contributing antioxidant activity [44]. Concomitantly, the difference in activity amongst compounds 13a-f, 15a-f and
16a-d was due to the difference in the stability of the oxygen centered radical formed in these compounds. Further, the results exemplified that the compounds 16a-d having the thiazole unit in combination with phenyl showed better radical scavenging activities than that of compounds 13a-d, 13f and 15a-e (see Table 1).

5-LOX inhibition activity

Compounds with antioxidant properties could be expected to offer protection in inflammation and to lead to potentially effective drugs. Hence, the synthesized compounds 13a-f, 15a-f and 16a-d were further evaluated for their inhibitory properties of 5-LOX enzyme assay described by Frum and Viljoen. The potency (IC$_{50}$ values) of test compounds was determined and compared to that of the reference molecule nordihydroguaiaretic acid (NDGA); these results are summarized in Table 2. The most active molecule 15f showed potent inhibition of 5-LOX with an IC$_{50}$ value of 10 µM (Table 2).

Perusal of IC$_{50}$ values shows that compound 15f is the most active, within the set, followed by compounds 16c and 13e. On the other hand, the compounds 13a-d, 15a-e and 16d showed IC$_{50}$ greater than >125 µM. Most LOX inhibitors are antioxidants or free radical scavengers [45]. The relationship between LOX inhibition and the ability of the inhibitors to chelate and reduce the active site Fe$^{3+}$, or compete with arachidonic acid for binding to the enzyme active site, are well known [46].

The introduction of methyl group on 4-position of phenyl ring decreased the activity. Compound 15f with a p-OH group demonstrates highly significant inhibition compared to the corresponding p-OCH$_3$ substituted derivatives. Derivative 16c, bearing OCH$_3$ group at the p-position of phenylthiazol ring, is the second most potent compound (IC$_{50}$ 23 µM). Compared to 16c, compound 13e with a p-OCH$_3$ group of phenyl ring demonstrated lower inhibition.
**Table 2: In-vitro 5-LOX enzyme inhibition assay data for the compounds 13a-f, 15a-f and 16a-d**

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-LOX IC$_{50}$ (µM)</th>
</tr>
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<tbody>
<tr>
<td>13a</td>
<td>&gt;125</td>
</tr>
<tr>
<td>13b</td>
<td>&gt;125</td>
</tr>
<tr>
<td>13c</td>
<td>&gt;125</td>
</tr>
<tr>
<td>13d</td>
<td>&gt;125</td>
</tr>
<tr>
<td>13e</td>
<td>41</td>
</tr>
<tr>
<td>13f</td>
<td>87</td>
</tr>
<tr>
<td>15a</td>
<td>&gt;125</td>
</tr>
<tr>
<td>15b</td>
<td>&gt;125</td>
</tr>
<tr>
<td>15c</td>
<td>&gt;125</td>
</tr>
<tr>
<td>15d</td>
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<tr>
<td>12</td>
<td>&gt;125</td>
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<tr>
<td>NDGA</td>
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</tr>
</tbody>
</table>

**CONCLUSION**

In conclusion, three new series of quinazolinone derivatives were synthesized and evaluated for antioxidant activity as well as for 5-LOX inhibition. All compounds showed antioxidant activity and analogs 13e, 15f and 16c demonstrated potent antioxidant activity. The compounds 13f and 13b showed moderate antioxidant activity. Synthesized compounds were evaluated for their 5-LOX inhibitory assay and compounds 15f and 15c exhibited significant inhibition. The in vitro 5-LOX activity of 15f and 16c seems to be related with their high radical scavenging activities. More specifically, it is evident that, compounds 15f and 16c exhibit satisfactory antioxidant and 5-lipoxygenase activities. Therefore, the design of this type of dual acting molecules should be further explored based on the structural features of these compounds.
Chapter 4
Quinazolinone derivatives

Figure 1: $^1$H NMR spectrum of compound 13a

Figure 2: $^1$H NMR spectrum of compound 13f
Figure 3: $^1$H NMR spectrum of compound 15f

Figure 4: $^1$H NMR spectrum of compound 16c
Figure 5: $^{13}$C NMR spectrum of compound 13f

Figure 6: $^{13}$C NMR spectrum of compound 15f
Figure 7: LC-MS of compound 13f

Figure 8: LC-MS of compound 15d
Figure 9: LC-MS of compound 16c
REFERENCES


Chapter 4

Quinazolinone derivatives


