

## Synthesis and study of new 4-quinazolinone inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP)

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**Dedicated to Prof. Gábor Bernáth on the occasion of his 70<sup>th</sup> birthday**

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### Abstract

4-Quinazolinones modified with 2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole or 2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridine rings and their *N*-oxyl derivatives were synthesized and some of them evaluated for protecting activity against H<sub>2</sub>O<sub>2</sub> induced cell death on WRL-68 human liver cell line. Compounds **15a**, **15c** and **15d** exhibited remarkably inhibitory effects on PARP enzyme *in vitro*.

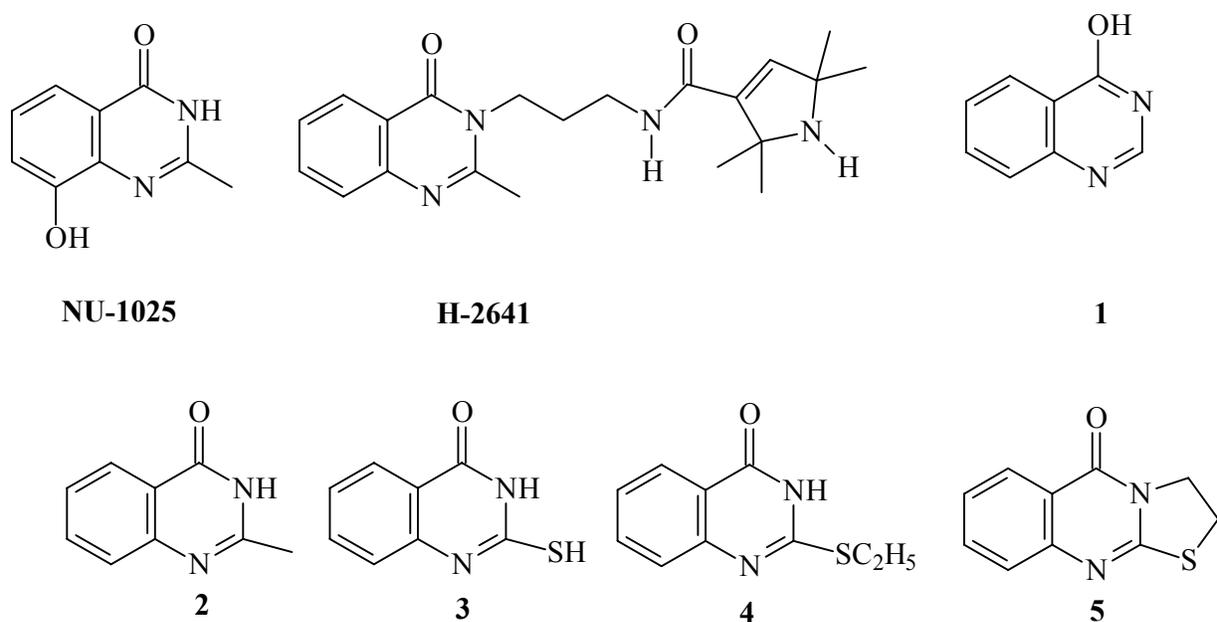
**Keywords:** Quinazolines, PARP inhibitors, nitroxides, free radical scavengers

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### Introduction

Poly(ADP-ribose) polymerase is an abundant eucariotic nuclear zinc-finger DNA-binding protein with a role of genomic repair.<sup>1,2</sup> DNA strand breaks generated either directly by genotoxic agents (alkylating agents, reactive oxygen species (ROS), ionizing radiation) or indirectly, following enzymatic incision of a DNA-base lesion, activate PARP enzyme. PARP catalyzes the biochemical conversion of the respiratory coenzyme, nicotinamide dinucleotide (NAD) to poly(ADP-ribose) and nicotinamide, therefore the latter is a weak feedback inhibitor of this enzyme. Although PARP has an important role in genomic repair mechanism, the massive activation of PARP results in extended ADP ribosylation and depletion of NAD. In the next step this leads to ATP depletion in an effort to resynthesize NAD and ultimately the lack of ATP and NAD causes mitochondrial dysfunction and cell death. The first PARP inhibitors were based on

mimicking the structure of nicotinamide and benzamide analogs.<sup>3</sup> Recent research aimed at synthesizing more potent and selective PARP inhibitors based on the crystal structure of the catalytic domain of PARP, leading to polycyclic amides and lactams.<sup>4,5</sup> One of the most potent compounds evaluated recently is the 8-hydroxy-2-methylquinazolin-4(3*H*)-one (**NU-1025**,  $IC_{50}$  = 400 nM).<sup>6,7</sup> In our laboratory we have synthesized 4-quinazolinone derivative, **H-2641** as an antiarrhythmic drug candidate.<sup>8</sup> It was a real challenge to test and compare this compound with basic quinazolines **1-3** (Figure 1) on PARP inhibitory activity assay as well as synthesizing new quinazolinone derivatives attached to 1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole or 1-oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridine and their amine precursors, because these rings exhibited radical scavenging activity and protected against oxidative stress.<sup>9,10</sup>



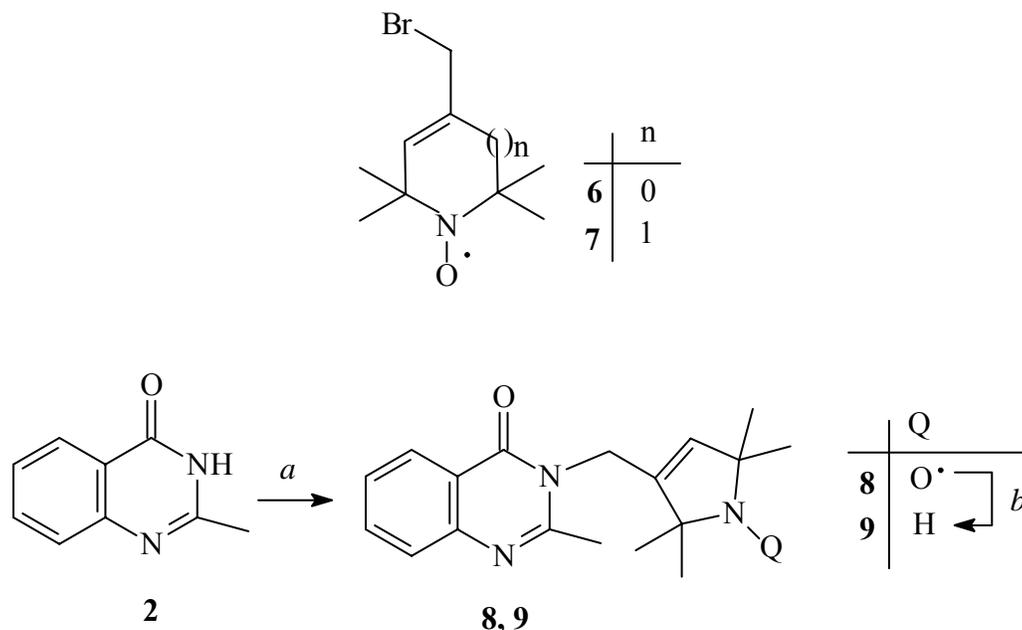
**Figure 1**

This fact can be a significant contribution in designing new PARP inhibitors against cell damage induced by antiviral<sup>11</sup> and anticancer drugs. Application of anticancer and antiviral compounds in the therapy is also mostly inevitably complemented by ROS formation whose *in statu nascendi* scavenging may moderate further the side-effects of these drugs compared to regular PARP inhibitors.

## Results and Discussion

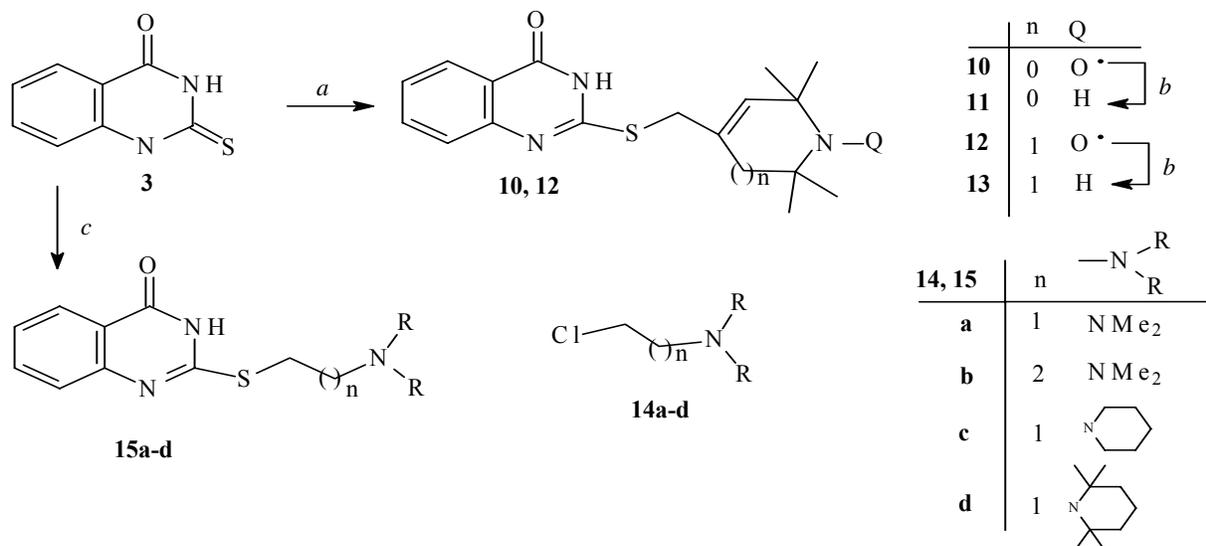
Compounds **4**<sup>12</sup> and **5**<sup>13</sup> could be obtained by alkylation of compound **3** with ethyl iodide and 1,2-dibromoethane, respectively. These compounds have not exhibited any PARP inhibitory

activity although compound **4** was reported to have antifungal activity, while compound **5** was reported to have an antihypertensive effect. To get paramagnetic derivatives we alkylated 2-methyl-4(3*H*)-quinazolinone (**2**) in DMF in the presence of  $K_2CO_3$  with 1-oxyl-3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole<sup>14</sup> (**6**) to give compound **8**. This could be reduced to secondary amine with Fe powder in glacial acetic acid<sup>15</sup> (Scheme 1).



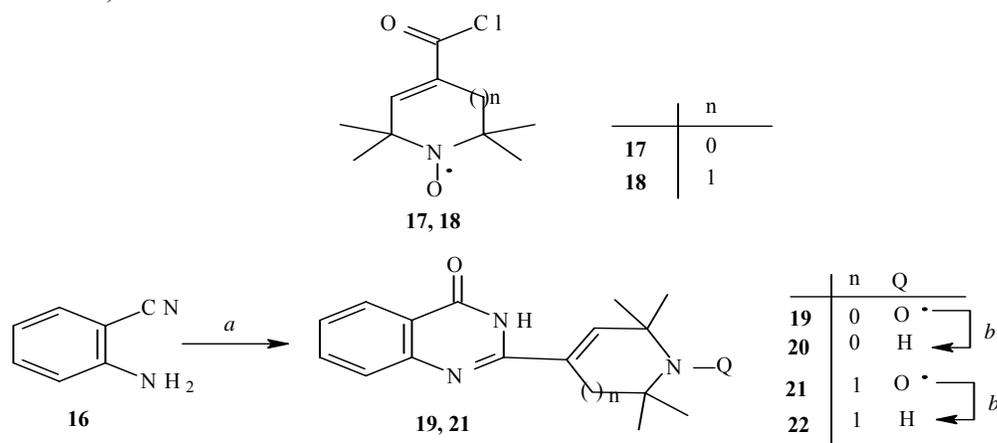
**Scheme 1.** Reagents and conditions: a) **6** (1 eq.),  $K_2CO_3$  (1 eq.), DMF, 90 °C, 6 h, 57 %; b) Fe, AcOH, 70 °C, 1 h, then  $K_2CO_3$  (42 %).

Unfortunately neither compound **8** nor **9** has significant PARP inhibitory activity (Table 1) in accordance with earlier reports showing that alkylation of lactam NH resulted in loss of PARP activity.<sup>7</sup> It was obvious that lactam must remain intact to get more active PARP inhibitors, so compound **3** was alkylated with five-membered (**6**) and six-membered (**7**)<sup>16</sup> paramagnetic allylic bromides to give compounds **10** and **12**, respectively. The corresponding amino derivatives **11** and **13** were achieved by reduction of paramagnetic compounds with Fe powder in AcOH. Compounds with amine side-chains were obtained by alkylation of compound **3** with similar, but more readily available amine derivatives such as 2-dimethylaminoethyl chloride **14a**, 3-dimethylaminopropyl chloride **14b**, 1-(2-chloroethyl)piperidine **14c** and 2,2,6,6-tetramethyl-1-(2-chloroethyl)piperidine **14d**<sup>17</sup> in DMF in the presence of 2 eq.  $K_2CO_3$  to afford compounds **15a-d**, respectively (Scheme 2). Tertiary amines **15a**, **15c** and **15d** exhibited better PARP inhibitory activity than secondary amines **11**, **13** or their corresponding radicals **10**, **12**.



**Scheme 2.** Reagents and conditions: a) **6** or **7** (1 eq.), K<sub>2</sub>CO<sub>3</sub> (1 eq.), DMF, 90 °C, 6 h, 44-58 %; b) Fe, AcOH, 70 °C, 1 h, then K<sub>2</sub>CO<sub>3</sub> 37-48 %; c) **14 a-d** (1 eq.), K<sub>2</sub>CO<sub>3</sub> (2 eq.), DMF, 90 °C, 8 h, 39-70 %.

Finally, our attention turned to such quinazolinone derivatives in which pyrroline and tetrahydropyridine rings were not linked *via* heteroatom. Considering that anthranilic acid and  $\beta$ -amino acid derivatives are readily available key starting materials for quinazoline synthesis,<sup>18</sup> this problem could be solved by acylation of 2-aminobenzonitrile with paramagnetic acid chlorides **17**<sup>19</sup> and **18** in CH<sub>2</sub>Cl<sub>2</sub> in the presence of triethylamine. The crude amides could be cyclised in aqueous dioxane with NaBO<sub>3</sub><sup>20</sup> to give compounds **19** and **21**, respectively. Reduction of compounds **19** and **21** with Fe powder in AcOH gave the corresponding amines **20** and **22** (Scheme 3).



**Scheme 3. Reagents and conditions:** a) **17** or **18** (1 eq.), Et<sub>3</sub>N (1 eq.), CH<sub>2</sub>Cl<sub>2</sub>, 1 h, 0 °C → r.t. then NaBO<sub>3</sub> · 4 H<sub>2</sub>O (2 eq.) dioxane/water, 100 °C, 7 h, 35-43 %; b) Fe, AcOH, 70 °C, 1 h, then K<sub>2</sub>CO<sub>3</sub>, 55-68 %.

Regarding the structure-activity relationship (SAR), the principal ring compound protecting activity greatly varied depending on the substituent. Compounds **1**, **2** exhibited good activity, compound **3** exhibited limited activity, **4**, **5** and **H-2641** with cardioprotective activity<sup>8</sup> were inactive in PARP inhibitory assay. The ω-amino *S*-alkylated compounds **15a-d** exhibited better protection activity than the *S*-ethyl one (**4**). The piperidine compounds **15c** and **15d** did not have any significant difference in activity, the preliminary toxicity was also similar. Among the dimethylamino containing side-chain compounds **15a** exhibited better activity than **15b**. Compounds substituted on the lactam NH (**8**, **H-2641**) were inactive, while compound **9** exhibited some moderate activity. Quinazolinones substituted at the 2-position with tetrahydropyridine rings showed good activity, however the amino precursor **22** had slightly better activity than the corresponding *N*-oxyl derivative **21**. The *N*-hydroxy salt of radical **19** was inactive (Table 1 and 2).

**Table 1.** Protecting effect of compounds (**H-2641**, **1-22**) against H<sub>2</sub>O<sub>2</sub> induced cell death determined in WRL-68 human liver cell line

Compound	<b>H-2641</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>8</b>	<b>9</b>
IC <sub>50</sub> (μM)	> 100	9	4	70	> 100	> 100	> 100	10
Maximal protection (%)	0	50	54	52	0	0	0	15
Compound	<b>13</b>	<b>15a</b>	<b>15b</b>	<b>15c</b>	<b>15d</b>	<b>19</b>	<b>21</b>	<b>22</b>
IC <sub>50</sub> (μM)	10	0.15	4.8	0.8	0.1	> 100	13	12
Maximal Protection (%)	61	68	65	62	69.6	0	50	50

**Table 2.** Inhibitory effects of compounds **15a-d** on PARP enzyme in vitro

Compound	<b>15a</b>	<b>15b</b>	<b>15c</b>	<b>15d</b>
IC <sub>50</sub> (μM)	2	6	2.6	1.6

In conclusion, series of 4(*3H*)-quinazolinone derivatives were synthesized by a convenient alkylation of 4(*3H*)-quinazolinones (**2**, **3**) and acylation of anthranilonitrile followed by

cyclisation. Among the synthesized and studied compounds 4(3*H*)-quinazolinone derivatives substituted at the 2-position with tertiary amines containing *S*-ethyl spacer were found to be effective PARP inhibitors. Nitroxides or its amino precursor has lower inhibitory activity than tertiary amines, however nitroxides and its amino precursor may have a potential advantage as a possible radical scavenger analogously to the previous results.<sup>9,10,21</sup>

## Experimental Section

**General Procedures.** Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyser. The IR (Specord 75) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode. <sup>1</sup>H NMR spectra were recorded with Varian Unity Inova 400 WB spectrometer; chemical shifts were referenced to TMS. Flash column chromatography was performed on Merck Kieselgel 60 (0.040-0.063 mm). The HPLC analyses were performed on HP1100, UV detection monitored at 250 nm using a Hypersyl BDS-C18 column. Qualitative TLC was carried out on commercially prepared plates (20 x 20 x 0.02 cm) coated with Merck Kieselgel GF<sub>254</sub>. Compounds **H-2641**,<sup>8</sup> **4**,<sup>12</sup> **5**,<sup>13</sup> **6**,<sup>14</sup> **7**,<sup>16</sup> **15d**,<sup>16</sup> **17**,<sup>18</sup> **18**<sup>18</sup> were prepared according to published procedures, compound **1**, **2**, **3**, 2-dimethylaminoethyl chloride, 3-dimethylaminopropyl chloride, 1-(2-chloroethyl)piperidine and anthranilonitrile were purchased from Aldrich.

### Protecting effect of 4-quinazolinone derivatives against H<sub>2</sub>O<sub>2</sub> induced cell death determined in WRL-68 human liver cell line

**Cell culture.** WRL-68 human liver cell line was from American Type Culture Collection (Rockville, MD). Cell lines were grown in humidified 5 % CO<sub>2</sub> atmosphere at 37 °C and maintained in culture as monolayer adherent cells in Dulbecco's Modified Eagle's Medium containing 1% antibiotic-antimycotic solution (Sigma, St. Louis, MO) and 10 % fetal calf serum. Cells were passaged at intervals of 3 days. *Detection of cell survival.* Cells were seeded into 96-well plates at a starting density of 2.5 x 10<sup>4</sup> cell/well and cultured overnight in humidified 5 % CO<sub>2</sub> atmosphere at 37 °C. The following day, 0.3 mM H<sub>2</sub>O<sub>2</sub> was added to the medium either alone or in the presence of protecting agent. Three hours later, the medium was removed and 0.5 % of the water soluble mitochondrial dye, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT<sup>+</sup>) was added. Incubation was continued for 3 more hours, the medium was removed, and the metabolically reduced water insoluble blue formazan dye was solubilised by acidic isopropanol. Optical densities were determined by an Anthos Labtech 2010 ELISA reader (Wien, Austria) at 550 nm wavelength. All experiments were run in at least 6 parallels and repeated 3 times. Data showed in Table 1 are the concentration in μM at which the rate of H<sub>2</sub>O<sub>2</sub> induced cell death is inhibited with 50 %.

### Inhibitory effects of 4-quinazolinone derivatives on PARP enzyme in vitro

The poly-ADP-ribose polymerase was isolated from rat liver based on the method described before.<sup>22,23</sup> The potential inhibitory effects of 4-quinazolinone derivatives were tested in this assay system. The PARP activity was determined in 130  $\mu$ l reaction mixture contained 100 mM Tris-HCl buffer, pH 8.0, 10 mM MgCl<sub>2</sub>, 10 % glycerol, 1.5 mM DTT, 1 mM [Adenine-2,8-<sup>3</sup>H] NAD<sup>+</sup> (4.500 cpm/nmol), 10  $\mu$ g activated DNA and 10  $\mu$ g histones. The incubation time was 15 minutes in the presence or in the absence of 4-quinazolinone derivatives, and the reaction was stopped by the addition of trichloroacetic acid (8 %). After addition of 0.5 mg albumin, precipitation was allowed to proceed for at least 20 minutes on ice, and the insoluble material was collected on a glass filter washed five times with 5 % perchloric acid. The protein-bound radioactivity was determined by a LS-200 Beckman scintillation counter, data showed in Table 2 are the concentration in  $\mu$ M.

**3-[(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl]-2-methylquinazolin-4(3H)-one radical (8).** A solution of 2-methylquinazolin-4(3H)-one (2) (1.60 g, 10.0 mmol), allylic bromide (6) (2.33 g, 10.0 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.38 g, 10.0 mmol) in DMF (15 mL) was stirred at 90 °C for 6 h. The solvent was evaporated, the residue was dissolved in CHCl<sub>3</sub> (30 mL), washed with brine (10 mL), the organic phase was separated, dried (MgSO<sub>4</sub>), filtered, evaporated and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound 1.77 g (57 %) as a yellow solid, mp 110-112 °C. Anal. calcd. for C<sub>18</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub>: C 69.21, H 7.10, N 13.45; Found: C 69.07, H 7.29, N 13.59. IR (nujol)  $\nu$ : 1620, 1585, 1570 cm<sup>-1</sup>. MS (m/z, %): 312 (M<sup>+</sup>, 6), 282 (21), 138 (75), 122 (100).

**Alkylation of 2-mercapto-4(3H)-quinazolinone. General procedure (10, 12, 15a-d).** A solution of 2-mercaptoquinazolin-4(3H)-one **3** (1.78 g, 10.0 mmol) and the corresponding alkyl halide **6** or **7** or **14a** or **14b** or **14c** or **14d** (10.0 mmol) and K<sub>2</sub>CO<sub>3</sub> (10.0 mmol or 20.0 mmol in case of **15a-d**) in DMF (25 mL) was stirred at 90 °C for 6 h. The solvent was evaporated off, the residue was dissolved in CHCl<sub>3</sub> (30 mL), washed with brine (10 mL), the organic phase was separated, dried (MgSO<sub>4</sub>), filtered, evaporated and the residue was purified by flash column chromatography (CHCl<sub>3</sub>/Et<sub>2</sub>O) to give the title compound **10** or **12** or **15a** or **15b** or **15c** or **15d**.

**2-[(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl]thio}quinazolin-4(3H)-one radical (10).** 1.91 g (58 %), mp 138-140 °C, yellow solid. Anal. calcd. for C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S: C 61.79, H 6.11, N 12.73, S 9.68; Found: C 61.92, H 6.30, N 12.79, S 9.60. IR (nujol)  $\nu$ : 1675, 1605, 1585, 1560 cm<sup>-1</sup>. MS (m/z, %): 330 (M<sup>+</sup>, 5), 300 (20), 178 (54), 122 (100).

**2-[(1-Oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-yl)methyl]thio}quinazolin-4(3H)-one radical (12).** 1.51g (44 %), mp 79-82 °C, pale orange solid. Anal. calcd. for C<sub>18</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub>S: C 62.76, H 6.44, N 12.21, S 9.29; Found: C 62.72, H 6.31, N 12.34, S 9.44. IR (nujol)  $\nu$ : 1670, 1610, 1585, 1560 cm<sup>-1</sup>. MS (m/z, %): 344 (M<sup>+</sup>, 6), 314 (35), 136 (61), 121 (100).

**2-[[2-(Dimethylamino)ethyl]thio}quinazolin-4(3H)-one (15a).** 1.29 g (52 %), mp 155-157 °C, white solid. Anal. calcd. for C<sub>12</sub>H<sub>15</sub>N<sub>3</sub>OS: C 57.81, H 6.06, N 16.85, S 12.86; Found: C 57.92, H 6.11, N 16.80, S 12.70. IR (nujol)  $\nu$ : 1665, 1630, 1570 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ <sub>H</sub>: 8.17 (1H, d),

7.65 (1H, td), 7.55 (1H, d), 7.35 (1H, t), 3.14 (2H, m), 2.92 (2H, m) and 2.48 (6H, s).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 163.4, 156.0, 149.3, 134.3, 126.3, 126.3, 125.7, 120.4, 60.9, 44.4, 30.1. MS ( $m/z$ , %): 249 ( $\text{M}^+$ , 2), 234 (16), 162 (44), 71 (100).

**2-[[3-(Dimethylamino)propyl]thio]quinazolin-4(3H)-one (15b).** 1.18 g (45 %), mp 93-95 °C, white solid. Anal. calcd. for  $\text{C}_{13}\text{H}_{17}\text{N}_3\text{OS}$ : C 59.29, H 6.51, N 15.96, S 12.17; Found: C 59.70, H 6.51, N 16.02, S 12.02. IR (nujol)  $\nu$ : 1665, 1630, 1570  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 8.16 (1H, d), 7.65 (1H, t), 7.54 (1H, d), 7.34 (1H, t), 3.23 (2H, t), 2.68 (2H, t), 2.38 (6H, s) and 1.96 (2H, m).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 163.8, 156.9, 149.1, 134.2, 126.3, 126.1, 125.5, 120.2, 54.9, 43.7, 28.7, 26.3. MS ( $m/z$ , %): 263 ( $\text{M}^+$ , 1), 205 (2), 85 (100), 58 (65).

**2-[(2-Piperidin-1-ylethyl)thio]quinazolin-4(3H)-one (15c).** 2.02 g (70 %), mp 131-133 °C, white solid. Anal. calcd. for  $\text{C}_{15}\text{H}_{19}\text{N}_3\text{OS}$ : C 62.26, H 6.62, N 14.53, S 11.06; Found: C 62.74, H 6.51, N 14.59, S 10.99. IR (nujol)  $\nu$ : 1660, 1640, 1575, 1550  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta_{\text{H}}$ : 12.82 (1H, bs), 8.02 (1H, d), 7.74 (1H, t), 7.49 (1H, d), 7.40 (1H, t), 3.34 (2H, t), 2.64 (2H, t), 2.46 (4H, m), 1.53 (4H, m) and 1.39 (2H, m).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta_{\text{C}}$ : 161.5, 156.4, 148.5, 134.5, 126.0, 125.7, 125.4, 120.0, 57.8, 53.8, 27.5, 25.2, 23.9. MS ( $m/z$ , %): 289 ( $\text{M}^+$ , 1), 178 (3), 111 (100), 98 (79).

**2-[[2-(2,2,6,6-Tetramethylpiperidin-1-yl)ethyl]thio]quinazolin-4(3H)-one (15d).** 1.34 g (39 %), mp 221-222 °C, white solid. Anal. calcd. for  $\text{C}_{19}\text{H}_{27}\text{N}_3\text{OS}$ : C 66.05, H 7.88, N 12.17, S 9.26; Found: C 66.15, H 7.85, N 12.29, S 9.10. IR (nujol)  $\nu$ : 1670, 1640, 1575, 1560  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta_{\text{H}}$ : 12.54 (1H, bs), 8.01 (1H, d), 7.74 (1H, t), 7.41 (1H, d), 7.38 (1H, t), 3.07 (2H, m), 2.77 (2H, m), 1.49 (2H, bs), 1.39 (4H, bs) and 1.10 (12H, s).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta_{\text{C}}$ : 161.3, 156.0, 148.5, 134.6, 126.1, 125.4, 125.4, 120.0, 54.3, 45.0, 40.6, 32.7, 17.2. MS ( $m/z$ , %): 345 ( $\text{M}^+$ , <1), 330 (1), 205 (16), 154 (100).

**Synthesis of 2-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)quinazolin-4(3H)-one radical (19) and 2-(1-Oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridine-4-yl)quinazolin-4(3H)-one radical (21).** To a stirred solution of 2-amino-benzonitrile (1.18 g, 10.0 mmol) and  $\text{Et}_3\text{N}$  (1.11 g, 11.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) freshly made compound **17** or **18** (10.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added dropwise at 0 °C, then the mixture was stirred further at rt. for 1 h. The organic phase was washed with aq. 10 %  $\text{K}_2\text{CO}_3$  (15 mL), aq. 5 %  $\text{H}_2\text{SO}_4$  (10 mL), brine (15 mL), the organic phase was separated, dried ( $\text{MgSO}_4$ ), filtered, evaporated. The crude amide was dissolved in dioxane (40 mL) and water (40 mL). The mixture was heated to reflux and treated with  $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$  (4.6 g, 30.0 mmol) over 2 h and the mixture was refluxed till consumption of the amide (5 h). After cooling the solvents were evaporated under reduced pressure, the residue was dissolved in  $\text{CHCl}_3$  (20 mL), washed with brine (10 mL), the organic phase was separated, dried ( $\text{MgSO}_4$ ), filtered, evaporated and the residue was purified by flash column chromatography ( $\text{CHCl}_3/\text{Et}_2\text{O}$ ) to give the title compound **19** as yellow solid 1.22 g (43 %), mp 224-226 °C. Anal. calcd. for  $\text{C}_{16}\text{H}_{18}\text{N}_3\text{O}_2$ : C 67.59, H 6.38, N 14.78; Found C 67.41, H 6.51, N 14.66. IR (nujol)  $\nu$ : 1650, 1600, 1570  $\text{cm}^{-1}$ , MS ( $m/z$ , %): 284 ( $\text{M}^+$ , 6), 270 (15), 254 (18), 41 (100). Compound **21** is an orange solid, 1.04 g (35 %), mp 226-228 °C. Anal. calcd. for

C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>: C 68.42, H 6.76, N 14.09; Found C 68.41, H 6.83, N 13.94. IR (nujol)  $\nu$ : 1670, 1600, 1580 cm<sup>-1</sup>, MS (m/z, %): 298 (M<sup>+</sup>, 10), 160 (23), 138 (48), 122 (100).

**Reduction of radicals to secondary amines; General procedure (9, 11, 13, 20, 22).** To a solution of nitroxide **8** or **10** or **12** or **19** or **21** (5.0 mmol) in AcOH (8 mL) Fe powder (1.40 g, 25 mmol) was added and the mixture was warmed up to 70 °C until the reaction started. The mixture was stirred at rt. for 1 h, diluted with water (20 mL), decanted and made alkaline with solid K<sub>2</sub>CO<sub>3</sub>. The mixture was extracted with CHCl<sub>3</sub> (3 x 15 mL), dried (MgSO<sub>4</sub>), filtered, evaporated and after chromatographic purification (CHCl<sub>3</sub>/MeOH) we got amines as white or off-white solids.

**3-[(2,2,5,5-Tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl]-2-methylquinazolin-4(3H)-one (9).** 623 mg (42 %), mp 95-97 °C, off-white solid. Anal. calcd. for C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O: C 72.68, H 7.80, N 14.14; Found C 72.57, H 7.82, N 14.21. IR (nujol)  $\nu$ : 3260, 1670, 1630, 1570, 1560 cm<sup>-1</sup>.

<sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$ <sub>H</sub>: 8.11 (1H, d), 7.90 (1H, t), 7.82 (1H, d), 7.61 (1H, t), 5.79 (1H, s), 5.11 (2H, s), 2.62 (3H, s), 1.29 (6H, s) and 1.18 (6H, s). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$ <sub>C</sub>: 165.5, 162.9, 151.0, 141.3, 135.0, 133.9, 126.8, 126.6, 123.0, 113.8, 65.8, 63.2, 62.3, 30.7, 30.0, 26.0.

MS (m/z, %): 297 (M<sup>+</sup>, 1), 282 (26), 160 (3), 122 (100).

**2-[(2,2,5,5-Tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl]thio}quinazolin-4(3H)-one (11).** 582 mg (37 %), mp 150-151 °C, white solid. Anal. calcd. for C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>OS: C 64.73, H 6.72, N 13.33, S 10.15; Found: C 64.76, H 6.63, N 13.23, S 10.02. IR (nujol)  $\nu$ : 3250, 1675, 1605, 1585, 1560 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ <sub>H</sub>: 8.16 (1H, d), 7.65 (1H, t), 7.52 (1H, d), 7.33 (1H, t), 5.60 (1H, s), 3.91 (2H, s), 1.29 (6H, s) and 1.15 (6H, s). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$ <sub>C</sub>: 162.1, 156.4, 148.6, 141.8, 134.2, 134.1, 126.0, 125.6, 125.2, 120.0, 66.8, 63.1, 30.5, 29.5, 25.8.

MS (m/z, %): 315 (M<sup>+</sup>, 1), 300 (26), 191 (14), 122 (100).

**2-[(2,2,6,6-Tetramethyl-1,2,3,6-tetrahydropyridin-4-yl)methyl]thio}quinazolin-4(3H)-one (13).** 789 mg (48 %), mp 180-182 °C, white solid. Anal. calcd. for C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>OS: C 65.62, H 7.04, N 12.76, S 9.71; Found: C 65.72, H 7.03, N 12.59, S 9.81. IR (nujol)  $\nu$ : 3260, 1670, 1610, 1585, 1570 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ <sub>H</sub>: 8.21 (1H, dd), 7.70 (1H, td), 7.56 (1H, d), 7.38 (1H, t), 5.74 (1H, s), 3.96 (2H, s), 2.03 (2H, s), 1.21 (6H, s) and 1.19 (6H, s). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$ <sub>C</sub>: 163.8, 155.4, 149.2, 134.6, 132.8, 127.7, 126.6, 126.2, 125.6, 120.0, 52.3, 50.7, 39.3, 38.0, 30.6, 29.4. MS (m/z, %): 329 (M<sup>+</sup>, 2), 314 (23), 136 (100), 121 (92).

**2-(2,2,5,5-Tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)quinazolin-4(3H)-one (20).** 914 mg (68 %), mp 254-256 °C, white solid. Anal. calcd. for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O: C 71.33, H 7.11, N 15.61; Found: C 71.38, H 7.06, N 15.60. IR (nujol)  $\nu$ : 3240, 1655, 1600, 1570 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$ <sub>H</sub>: 12.01 (1H, bs), 8.10 (1H, dd), 7.79 (1H, td), 7.62 (1H, d), 7.49 (1H, td), 6.96 (1H, s), 1.51 (6H, s) and 1.24 (6H, s). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$ <sub>C</sub>: 161.7, 148.8, 148.4, 144.6, 139.6, 134.4, 127.6, 126.6, 125.7, 121.1, 66.9, 63.2, 30.6, 30.3. MS (m/z, %): 269 (M<sup>+</sup>, 1), 254 (24), 137 (40), 108 (100).

**2-(2,2,6,6-Tetramethyl-1,2,3,6-tetrahydropyridine-4-yl)quinazolin-4(3H)-one (22).** 816 mg (55 %), mp 265-268 °C, white solid. Anal. calcd. for C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O: C 72.04, H 7.47, N 14.84; Found: C 72.21, H 7.53, N 14.64. IR (nujol)  $\nu$ : 3260, 1670, 1600, 1580 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-

$d_6$ )  $\delta_H$ : 12.01 (1H, bs), 8.10 (1H, d), 7.78 (1H, t), 7.64 (1H, d), 7.47 (1H, t), 6.89 (1H, s), 2.35 (2H, s), 1.23 (6H, s) and 1.13 (6H, s).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta_C$ : 161.9, 152.9, 148.6, 134.4, 127.3, 126.8, 126.3, 125.8, 125.8, 121.1, 51.8, 49.3, 36.3, 30.7, 29.6. MS (m/z, %): 283 ( $\text{M}^+$ , 8), 268 (75), 138 (52), 122 (100).

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