# Functionalization of Quinazolin-4-Ones Part 2[#]: Reactivity of 2-Amino-3, 4, 5, or 6-Nitrobenzoic Acids with Triphenylphosphine Thiocyanate, Alkyl Isothiocyanates, and Further Derivatization Reactions

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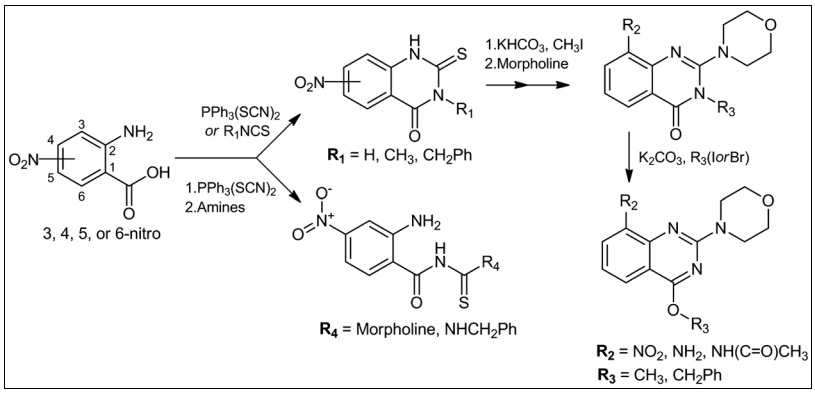
Additional Supporting Information may be found in the online version of this article.
Received December 18, 2013
DOI 10.1002/jhet.2235
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).




2-amino-3, 4, 5, or 6-nitrobenzoic acids were reacted with $PPh_3(SCN)_2$ and alkyl isothiocyanates to give 5, 6, 7, or 8-nitro-2-thioxo-3-substituted quinazolin-4-ones, respectively. The position of the nitro group was found to have significant influence on the outcome of the reactions. Similarly, the nature of the substituent at position 8 ($NO_2$, $NH_2$, $NH(C{=}O)CH_3$) in 8-substituted-2-methylthio quinazolin-4-ones was also found to significantly influence their reactivity towards morpholine. A selection of the products were also tested for *in vitro* antibacterial activity but little activity was observed.

*J. Heterocyclic Chem.*, **00,** 00 (2014).

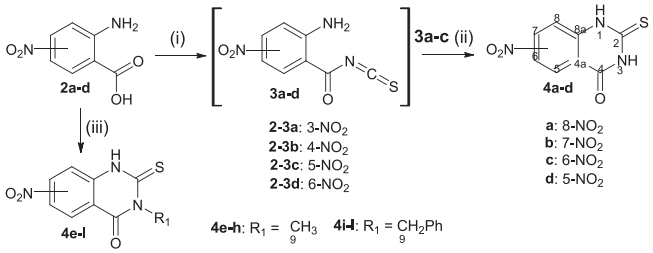
## INTRODUCTION

Quinazolines form a large family of compounds that are well recognized for their ability to show a broad range of biological activities, which are influenced significantly by the type of substituents and where they are placed around the quinazoline structure [1]. Observed biological activities include, but are not limited to, CNS depressant [1,2], anti-inflammatory [1,3–5], diuretic [1], antihypertensive [1], analgesic [4], anticancer [6–8], antihypoxic [9], hypoglycemic [10], and antimicrobial [11–15] activities. The ability to synthesize and functionalize quinazolines is therefore of great importance, and the methods employed will reflect the structure of the desired quinazolines. Synthesis of 7, or 8-substituted-2-morpholino quinazolines from 7, or 8-substituted-2-thioxo quinazolin-4-ones forms the continuum of this research for the development of potential DNA-PK and PI3K inhibitors, and antiplatelet compounds, which have been observed for the chromone and benzoxazine analogues [16–18]. There are many reported methods for 2-thioxo quinazolin-4-one synthesis in the literature; some of which can possess inherent limitations to functionalization and requires harsh conditions [19–21]. Recently, triphenylphosphine thiocyanate ($PPh_3(SCN)_2$) has been successful in cyclizing 2-aminobenzoic acid and 2-(*N*-methylamino)benzoic acid to their corresponding 2-thioxo quinazolin-4-ones in good yields [22]. This method is cheap and does not require harsh conditions but must be performed under strictly anhydrous conditions. In continuation of our previous work, we are reporting on the reactivity of 2-amino-3, 5, or 6-nitrobenzoic acids with triphenylphosphine thiocyanate ($PPh_3(SCN)_2$) and alkyl isothiocyanates (RNCS), which are two reagents employed in the synthesis of substituted 2-thioxo quinazolin-4-ones from substituted 2-aminobenzoic acids. Additionally, the synthesis of 8-(nitro and amino)-2-morpholine quinazolines is presented and its reactivity compared with our previously reported work regarding 7-nitro quinazolines [23].

## RESULTS AND DISCUSSION

**Synthesis of 8, 7, 6, or 5-nitro-2-thioxo-3-substituted quinazolin-4-one (4a-l).** Previously, we reported that compound **3b** (4-$NO_2$) was isolated from the reaction of compound **2b** (4-$NO_2$) with $PPh_3(SCN)_2$ [23]. In continuation of our investigation, 2-amino-3, 5, or 6-nitrobenzoic acid (**2a**, **2c–d**), synthesized from 6, 5, 4, or 3-nitro-2-methylanilines (**1a–d**) (see Supporting Information for details), were likewise reacted under the same conditions to determine the stability of their benzoyl isothiocyanate intermediates (**3a**, **3c–d**) (Scheme 1).

**Scheme 1.** Synthesis of 5, 6, 7 or 8-nitro-2-thioxo quinazolin-4-ones. (i) = $PPh_3(SCN)_2$, 0°C to RT, reflux 16–20 h. (ii) = $K_2CO_3$, acetonitrile, reflux 1.5 h. (iii) = $R_1NCS$, TEA, ethanol, reflux, 6 h or $R_1NCS$, TEA, acetonitrile, mw, 140°C, 2 h, P = 60–100 W.




| Product | $NO_2$ pos. | Yield (%): Thermal (Microwave) | Product | $NO_2$ pos. | Yield (%): Thermal (Microwave) |
|---|---|---|---|---|---|
| 4a | 8 | 82 (-) | 4g | 6 | 10 -56 |
| 4b | 7 | 76* (-) | 4h | 5 | 90 (-) |
| 4c | 6 | 48 (-) | 4i | 8 | NR -35 |
| 4d | 5 | NR (-) | 4j | 7 | 87* (-) |
| 4e | 8 | NR (NR) | 4k | 6 | 16 -67 |
| 4f | 7 | 89* (-) | 4l | 5 | 90 (-) |

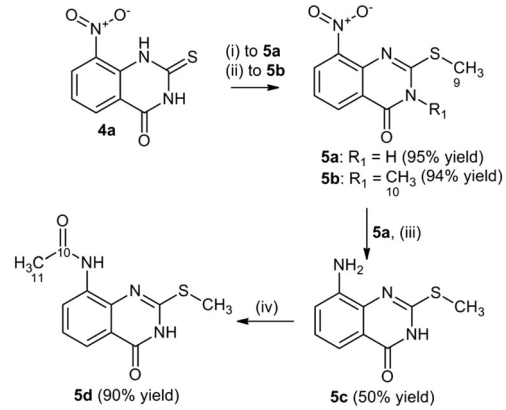
NR = No Reaction
(-) = Reaction not performed under microwave conditions.
* Values taken from reference 23.

Each of the compound **2a–d** behaved in a different manner when reacted with $PPh_3(SCN)_2$. Compound **2a** (3-$NO_2$) formed the corresponding 8-nitro-2-thioxo quinazolin-4-one (**4a**). Compound **2b** (4-$NO_2$), as previously reported [23], yielded benzoyl isothiocyanate **3b**. Compound **2c** (5-$NO_2$) gave a crude product containing ~80% of the intermediate benzoyl isothiocyanate **3c** and ~20% product **4c** as estimated by $^1$H-NMR. However, compound **2d** (6-$NO_2$) did not give any identifiable product. The cyclization of the benzoyl isothiocyanate intermediates **3b** and **3c** to **4b** and **4c,** respectively, was achieved by refluxing with potassium carbonate ($K_2CO_3$) in acetonitrile followed by acidification with HCl.

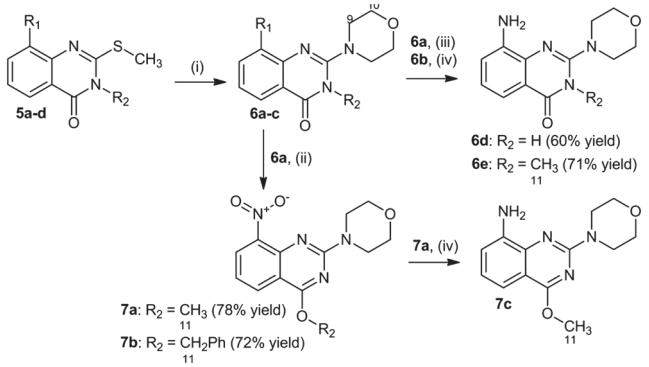
The reaction of 2-aminobenzoic acids with alkyl isothiocyanates is a well-established method for the synthesis of 2-thioxo-3-substituted quinazolin-4-ones (Scheme 2) [24]. The outcome of the reaction of 2-aminobenzoic acids **2a–d** with methyl and benzyl isothiocyanate was found to be dependent on the position of the nitro group, the alkyl isothiocyanate employed, and the reaction conditions. When the nitro group was *meta* to the amino group, as seen in compounds **2b** (4-$NO_2$) and **2d** (6-$NO_2$), the reaction with methyl and benzyl isothiocyanate proceeded easily under thermal reflux to give compounds **4f** (7-$NO_2$, $R_1 = CH_3$), **4j** (7-$NO_2$, $R_1 = CH_2Ph$), **4h** (5-$NO_2$, $R_1 = CH_3$), and **4l** (5-$NO_2$, $R_1 = CH_2Ph$) in high yields. However, if the nitro group was positioned *ortho*/*para* as seen in compounds **2a** (3-$NO_2$) and **2c** (5-$NO_2$), little to no reaction was observed. Compound **2a** (3-$NO_2$) reacted only with benzyl isothiocyanate under microwave conditions to give compound **4i** (8-$NO_2$, $R_1 = CH_2Ph$); thermal reflux with methyl or benzyl isothiocyanate yielded no product. Compound **2c** (5-$NO_2$) successfully reacted with methyl and benzyl isothiocyanate under thermal reflux to give **4g** (6-$NO_2$, $R_1 = CH_3$) and **4k** (6-$NO_2$, $R_1 = CH_2Ph$), respectively, in low yields (10% and 16%, respectively), which was improved upon under microwave conditions (56% and 67%, respectively) (Schemes 3 and 4).

**Synthesis of 8-substituted-2-morpholino quinazolines (6a–e, 7a–c).** It is known that 2-amino quinazolin-4-ones can be

**Scheme 2.** Synthesis of 8-substituted-2-methylthio quinazolin-4-ones. (i) = $KHCO_3$, $CH_3I$, Acetone, reflux, 1.5 h. (ii) = $K_2CO_3$, $CH_3I$, acetone, reflux, 1.5 h. (iii) = Fe, AcOH, ethanol, reflux 1.5 h *or* $SnCl_2.2H_2O$, ethylacetate, reflux, 8 h. (iv) = $Ac_2O$, AcOH, reflux, 30 min.



**Scheme 3.** Synthesis of 8-amino-2-morpholino quinazolines. (i) = morpholine, reflux, 3–72 h. (ii) = $K_2CO_3$, $CH_3I$ *or* benzyl bromide, acetone, reflux, 1.5 h. (iii) = $SnCl_2.2H_2O$, ethylacetate, reflux, 8 h. (iv) = Fe, HCl, ethanol, reflux 1.5 h.




| Starting Material | Product | $R_1$ | $R_2$ | Reaction Time (h) | Yield (%) |
|---|---|---|---|---|---|
| 5a | 6a | $NO_2$ | H | 3 | 77 |
| 5b | 6b | $NO_2$ | $CH_3$ | 8 | 77 |
| 5c | 6d | $NH_2$ | H | ~72 | trace |
| 5d | 6c | $NH(C{=}O)CH_3$ | H | ~72 | 58 |

synthesized by treating 2-alkylthio quinazolin-4-ones with amines [2]. From our previous investigations, it was shown that substituents on the 7 position ($NO_2$, $NH_2$, $NH(C{=}O)CH_3$) did not affect the susceptibility of the methylthio group toward morpholine [23]. The 8 position, however, is likely to exert a different electronic effect. The 2-methylthio compounds **5a** ($R_1 = H$) and **5b** ($R_1 = CH_3$) were readily synthesized by refluxing in acetone with $KHCO_3$ and $K_2CO_3$, respectively, and $CH_3I$. Reduction of compound **5a** ($R_1 = H$) with $SnCl_2.2H_2O$ gave **5c**, which was subsequently acetylated to give compound **5d** (Scheme 2).

Substituents on the 8 position were found to significantly affect the reactivity of the 2-methylthio compounds **5a–d** with morpholine (Scheme 3). Compound **5a** ($R_1 = 8\text{-}NO_2$, $R_2 = H$) reacted readily when refluxed in neat morpholine and was complete after 3 h compared with the 8 h required for the previously reported 7-nitro analogue [23]. Compound **5c** ($R_1 = 8\text{-}NH_2$, $R_2 = H$) did not yield any substantial amount of product even after 3 days of refluxing in neat morpholine. Compound **5d** ($R_1 = 8\text{-}NH(C{=}O)CH_3$, $R_2 = H$) reacted to completion only after 3 days of refluxing in neat morpholine as was determined by thin layer chromatography; microwave reflux did not improve the outcome. Additionally, the 2-methylthio group of the *N*3 methyl compound **5b** ($8\text{-}NO_2$, $R_2 = CH_3$) was easily replaced by morpholine to yield compound **6b** ($8\text{-}NO_2$, $R_2 = CH_3$) without the need to convert it to a sulfoxide as was required for the previously reported $7\text{-}NO_2$ analogue of **5b** [23].

The 8-nitro group can thus prove useful as it facilitates the synthesis of 2-amino quinazolines by increasing the susceptibility of the 2-methylthio group toward secondary amines.

The nitro group can then be converted to other functional groups such as an amino, which can then undergo many reactions typical of the primary aromatic amines. Further alkylation of **6a** ($R_1 = NO_2$, $R_2 = H$), according to the previously reported procedure [23], with $K_2CO_3$ and methyl iodide or benzyl bromide gave 4-*O*-alkylated products **7a–b**. Compound **6d** ($R_2 = H$) was prepared in good yields (60%) by reduction of compound **6a** ($R_1 = NO_2$, $R_2 = H$) using $SnCl_2.2H_2O$. In contrast, reduction by iron and hydrochloric acid in ethanol was preferred for the production of compounds **6e** ($R_2 = CH_3$) and **7c** from **6b** ($R_1 = NO_2$, $R_2 = CH_3$) and **7a** ($R_2 = CH_3$), respectively, as they were both stable to strong acids, the method was faster, and generally gave higher yields and purer products.

Structures of all the newly synthesized compounds were confirmed by analysis of $^1H$ and $^{13}C$-NMR spectroscopy in addition to Fourier transform infrared and microanalysis (see Supporting Information for all $^1H$ and $^{13}C$ spectra).

**Synthesis of 2-amino-N-(substituted(carbono or carbamo) thioyl)-4-nitrobenzamides (8a–b).** As isothiocyanates (R-NCS) undergo addition reactions with amines, the benzoyl isothiocyanate intermediate, **3b**, was treated with benzylamine and morpholine to give compounds **8a** and **8b**, respectively (Schemes 4). Further reactions, discussions, and experimental details are recorded in the Supporting Information.

**Antibacterial properties.** The antibacterial properties of quinazolin-4-ones have been widely reported in the scientific literature [14,25–28]. To assess the antibacterial properties of the newly synthesized quinazolines, disk diffusion assays were performed against the bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis* with Ciprofloxacin and Penicillin G potassium salt as positive controls (Table 1).

**Scheme 4.** Reaction of 2-amino-4-nitrobenzoyl isothiocyanate **3b** with aliphatic amines. (i) = aliphatic amine, dioxane, 50°C, 20 min.

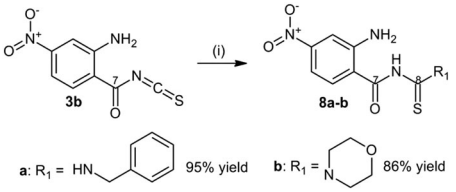



**Table 1**

Antibacterial data of the newly synthesized compounds.

| Disk diffusion assay diameter of inhibition zone (mm) | | |
|---|---|---|
| No. | *B. subtilis* | *S. aureus* |
| **4a** | 10 | 10 |
| **4b** | 8 | – |
| **4c, g-i, l** | – | – |
| **4f** | – | 11 |
| **4j** | 8 | 9 |
| **4 k** | – | 13 |
| **6a–e, 7a–c** | – | – |
| **8a** | 13 | 8 |
| **8b** | 14 | 9 |
| Ciprofloxacin | 37 | 28 |
| Penicillin G (K salt) | 33 | 23 |

Minus sign (–) = no zone of inhibition was observed around the 6 mm blank susceptibility disks.

Compared with the positive controls, the tested compounds showed very limited antibacterial activity. All the 7-nitro-2-thioxo quinazolin-4-ones **4b, f, and j** showed slight antibacterial activity against one of or both the gram-positive bacteria *B. subtilis* and *S. aureus*. Activity was also shown with compound **4a** (8-$NO_2$) but diminished with addition of the benzyl group at position 3 (**4i**) unlike compound **4k** (6-$NO_2$), which showed improved activity upon addition of a benzyl group. None of the 2-morpholino compounds (**6a–e, 7a–c**) showed antibacterial activity, and none of the tested compounds were active against *E. coli*, *P. aeruginosa*, and *E. faecalis*.

## CONCLUSION

$PPh_3(SCN)_2$ has shown to be a useful reagent for 2-amino-3, 4, or 5-nitrobenzoic acids but failed to react with 6-nitro-2-aminobenzoic acid. Additionally, benzyl isothiocyanate successfully reacted with 2-amino-3, 4, 5, or 6-nitrobenzoic acids under thermal and/or microwave conditions. However, methyl isothiocyanate only reacted with 2-amino-4, 5, or 6-nitrobenzoic acids. The reactivity of 8-substituted-2-methylthio quinazolin-4-ones toward morpholine was also found to be influenced significantly by the nature of the substituent at the 8 position; this was not the case with their previously reported 7-substituted analogues [23].

## EXPERIMENTAL

**Chemistry.** Infrared spectra were obtained using a Perkin Elmer FT-IR 1720× spectrometer (Perkin Elmer Ltd., Buckinghamshire, Seer Green, UK). $^1$H-NMR and $^{13}$C-NMR spectra were obtained using a Bruker AC 200 NMR spectrometer (Bruker, Banner Lane, Coventry, UK) at 200 and 50 MHz, respectively. All $^1$H-NMR and $^{13}$C-NMR spectral results are recorded as chemical shifts (δ) relative to the internal TMS for proton and 77.0 ppm in $CDCl_3$ solvent and 39.5 ppm in DMSO-$d_6$ solvent for $^{13}$C-NMR. $^1$H-NMR multiplicities are expressed as singlet (s), broad singlet (bs), doublet (d), double doublet (dd), triple doublet (td), triplet (t), double triplet (dt), quartet (q), multiplet (m), and broad multiplet (bm). Microanalysis was performed by Chemical and Microanalytical Services, Australia. Melting point determinations were carried out using a Stuart Scientific melting point apparatus and all melting points are uncorrected. All microwave reactions were carried out in a Milestone Microwave Lab Station. All starting materials were purchased from major chemical companies (Sigma Aldrich, Alfa Aesar, Merck) and used without any further purification. All solvents were purchased as laboratory grade and were used without further purification unless otherwise stated. Dichloromethane was dried over and distilled from calcium hydride and stored over 0.4nm molecular sieves.

**Synthesis of 2-amino-3, 4, 5, or 6-nitrobenzoic acids (2a–d)**

***General procedure A: synthesis of 2a–d.*** Synthesis of 2-amino-3, 4, 5, or 6-nitrobenzoic acids (**2a–d**) from 6, 5, 4, or 3-nitro-2-methylanilines, respectively, was performed according to a previously reported procedure [23] except for 6-nitro-2-aminobenzoic acid (**2d**) whereby the hydrolysis of the acetylamino group was performed using 70 mL of 50% (v/v) $H_2SO_4$ at 50°C for 16 h followed by neutralization with NaOH then filtration of the precipitated product **2d** (see Supporting Information for experimental details).

**Synthesis of 8, 7, 6, or 5-nitro-2-thioxo-3-substituted quinazolin-4-one (4a–d)**

***General procedure B: synthesis of triphenylphosphine thiocyanogen ($PPh_3(SCN)_2$).*** $PPh_3(SCN)_2$ (6.0 mmol) was freshly prepared *in situ* according to a previously reported procedure [23].

***General procedure C: reaction of 2-amino-3, 4, or 5-nitrobenzoic acids (2a-c) with $PPh_3(SCN)_2$ and cyclization of stable benzoyl isothiocyanate intermediates 3b and 3c.*** Products **2a** and **2b** (4.8 mmol) were reacted with freshly prepared $PPh_3(SCN)_2$ according to the previously reported procedure [23] to give **4a** and **3b**. Product **2c** was reacted according to a modified version of the same procedure in which the reaction mixture was allowed to stir at 0°C for 8 h, warm up to room temperature overnight then refluxed for 24 h to give a mixture of **3c**(80%) and **4c**(20%). Product **3b** and the crude mixture of **3c**(80%)/**4c**(20%) were then reacted with $K_2CO_3$ in acetonitrile according to the previously reported procedure to give **4b** and **4c**, respectively [23].

***8-Nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4a).*** Yellow solid. Yield: 3.9 mmol (82%). Solvent: acetonitrile. Mp 246–247° C. IR (KBr): 3319.7 (NH), 1693.5 (C=O), 1509.8 (s, $NO_2$), 1313.0 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 7.49 (t, $J_{6-5} = J_{6-7} = 7.8$ Hz, 1H, H-6), 8.34 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.2$ Hz, 1H, H-5), 8.54 (dd, $J_{7-6} = 8.0$ Hz, $J_{7-5} = 1.2$ Hz, 1H, H-7), 11.34 (bs, 1H, NH), 12.93 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 118.4, 123.4, 131.2, 133.3, 134.0, 134.2, 157.7, 174.5 ppm. *Anal*. Calcd for $C_8H_5N_3O_3S$ (223.21): C, 43.05; H, 2.26; N, 18.83. Found: C, 43.11; H, 2.31; N, 19.01.

***2-Amino-4-nitrobenzoyl isothiocyanate (3b).*** Yellow solid with identical physical and spectroscopic data to those previously reported [23]. Yield = 82%.

***7-Nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4b).*** Yellow solid with identical physical and spectroscopic data to those previously reported [23]. Yield = 93% from **3b** and 76% from **2b**.

***6-Nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4c).*** Light yellow solid. Yield: 2.3 mmol (48%). Solvent: ethanol. Mp 309–310°C (lit. mp = 267–268°C) [29]. IR (KBr): 3324.0 (m, NH), 3221.0 (m, NH), 1671.7 (s, C═O), 1341.0 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 7.50 (d, $J_{8-7}$ = 9.0 Hz, 1H, H-8), 8.47 (dd, $J_{7-8}$ = 9.0 Hz, $J_{7-5}$ = 2.6 Hz, 1H, H-7), 8.58 (d, $J_{5-7}$ = 2.6 Hz, 1H, H-5), 12.8 (bs, 2H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 116.2, 117.0, 122.5, 129.5, 142.7, 144.2, 158.2, 175.4 ppm. *Anal*. Calcd for $C_8H_5N_3O_3S$ (223.21): C, 43.05; H, 2.26; N, 18.83. Found: C, 43.30; H, 2.49; N 18.80.

**Synthesis of 3-substituted-8, 7, 6, or 5-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4f-l)**

***General procedure D: reaction of 2-amino-3, 4, 5, or 6-nitrobenzoic acids (2a–d) with alkylisothiocyanates under thermal conditions.*** Products **2b–d** (5.0 mmol) was reacted with methyl and benzyl isothiocyanate (6.0 mmol) according to previously reported procedure [24] to give **4f–h** and **4j–l**, respectively. The products were used without any further purification but can be purified by an appropriate technique. Product **2d** required a modified version in which the reaction was allowed to proceed at 50°C for 24 h to give **4h** and **4l**.

***General procedure E: reaction of 2-amino-3, 4, 5 or 6-nitrobenzoic acids with alkylisothiocyanates under microwave conditions.*** Products **2c** or **2a** (5.0 mmol), methyl, or benzyl isothiocyanate (10.0 mmol), triethylamine (10.0 mmol) were suspended in 10 mL acetonitrile in a 30 mL quartz tube and placed in a 40 bar sealed housing. The reaction mixture was placed in a Milestone Microwave Lab Station and heated to 140°C for 2 h while sustaining a power level of 60–100 W. The acetonitrile was then evaporated under reduced pressure, and the resulting solid was triturated with minimal ether then collected by filtration with suction to give **4g**, **4k**, and **4i**, respectively. The product was used without any further purification but can be purified by an appropriate technique.

***3-Methyl-7-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4f).*** Yellow solids with identical physical and spectroscopic data to those previously reported [23]. Yield = 89%.

***3-methyl-6-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4g).*** Light yellow solid. Yield: 0.5 mmol (10%) and 2.8 mmol (56%) from general procedures D and E, respectively. Solvent: ethanol. Mp 306–307°C (decomp.) IR (KBr): 3243.0 (m, NH), 1668.4 (s, C = O), 1630.1 (s, NH bd), 1551.5 (s, $NO_2$), 1340,1 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 3.67 (s, 3H, H-9), 7.52 (d, $J_{8-7}$ = 9.0 Hz, 1H, H-8), 8.48 (dd, $J_{7-8}$ = 9.0 Hz, $J_{7-5}$ = 2.4 Hz, 1H, H-7), 8.62 (d, $J_{5-7}$ = 2.4 Hz, 1H, H-5), 13.19 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 33.1, 115.1, 116.8, 123.1, 129.4, 142.6, 142.7, 158.3, 176.2 ppm. *Anal*. Calcd for $C_9H_7N_3O_3S$ (237.24): C, 45.57; H, 2.97; N, 17.71. Found: C, 45.63; H, 3.06; N, 17.76.

***3-Methyl-5-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4h).*** White solid. Yield: 4.5 mmol (90%). Solvent: nitromethane. Mp 275–276°C. IR (KBr): 3168.5 (m, NH), 1690.5 (s, C═O), 1622.9 (m, NH bd), 1547.4 (s, $NO_2$), 1386.1 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 3.61 (s, 3H, H-9), 7.52–7.60 (m, 2H, H-6, H-8), 7.86 (t, $J_{7-6}$ = $J_{7-8}$ = 7.8 Hz, 1H, H-7), 13.12 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 33.0, 105.9, 117.4, 118.0, 135.5, 139.7, 148.5, 155.9, 175.4 ppm. *Anal*. Calcd for $C_9H_7N_3O_3S$ (237.24): C, 45.57; H, 2.97; N, 17.71. Found: C, 45.51; H, 3.04; N, 17.69.

***3-Benzyl-8-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4i).*** Pale yellow solid. Yield: 1.75 mmol (35%). Solvent: diethyl carbonate. Mp 188–189°C. IR (KBr): 3334.9 (m, NH), 1685.1 (s, C═O), 1625.2 (m, NH bd), 1499.5 (s, $NO_2$), 1313.3 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 5.68 (s, 2H, H-9), 7.25–7.40 (m, 5H, benzylic hydrogens), 7.53 (t, $J_{6-5}$ = $J_{6-7}$ = 8.0 Hz, 1H, H-6), 8.41 (dd, $J_{5-6}$ = 8.0 Hz, $J_{5-7}$ = 1.4 Hz, 1H, H-5), 8.59 (dd, $J_{7-6}$ = 8.0 Hz, $J_{7-5}$ = 1.4 Hz, 1H, H-7), 11.69 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 49.1, 117.7, 123.6, 126.9, 127.0, 127.9, 131.5, 132.9, 133.2, 134.9, 135.4, 157.6, 175.4 ppm. *Anal*. Calcd for $C_{15}H_{11}N_3O_3S$ (313.33): C, 57.50; H, 3.54; N, 13.41. Found: C, 57.47; H, 3.62; N, 13.38.

***3-Benzyl-7-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4j).*** Yellow solid with identical physical and spectroscopic data to those previously reported [23]. Yield = 87%.

***3-Benzyl-6-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4k).*** Light yellow solid. Yield: 0.8 mmol (16%) and 3.35 mmol (67%) from general procedures D and E, respectively. Solvent: nitromethane. Mp 267–268°C. IR (KBr): 3280.9 (m, NH), 1670.1 (s, C═O), 1344.4 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 5.67 (s, 2H, H-9), 7.23–7.38 (m, 5H, benzylic hydrogens), 7.57 (d, $J_{8-7}$ = 9.0 Hz, 1H, H-8), 8.51 (dd, $J_{7-8}$ = 9.0 Hz, $J_{7-5}$ = 2.4 Hz, 1H, H-7), 8.64 (d, $J_{5-7}$ = 2.4 Hz, 1H, H-5), 13.31 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 48.7, 115.3, 117.0, 123.3, 126.7, 127.0, 127.9, 129.6, 142.8, 142.9, 158.2, 176.3 ppm. *Anal*. Calcd for $C_{15}H_{11}N_3O_3S$ (313.33): C, 57.50; H, 3.54; N, 13.41. Found: C, 57.43; H, 3.60; N, 13.57.

***3-Benzyl-5-nitro-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4l).*** White solid. Yield: 4.5 mmol (90%). Solvent: methanol. Mp 242–243°C. IR (KBr): 3182.0 (m, NH), 1708.0 (C═O), 1621.0 (s, NH bd), 1541.9 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 5.62 (s, 2H, H-9), 7.23–7.36 (m, 5H, benzylic hydrogens), 7.57 (dd, $J_{8-7}$ = 8.0 Hz, $J_{8-6}$ = 1.0 Hz, 1H, H-8), 7.62 (dd, $J_{6-7}$ = 8.0 Hz, $J_{6-8}$ = 1.0 Hz, 1H, H-6), 7.89 (t, $J_{7-6}$ = $J_{7-8}$ = 8.0 Hz, 1H, H-7), 13.26 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 48.7, 106.1, 117.7, 118.2, 126.7, 127.0, 127.9, 135.7, 135.8, 139.9, 148.5, 155.8, 175.6 ppm. *Anal*. Calcd for $C_{15}H_{11}N_3O_3S$ (313.33): C, 57.50; H, 3.54; N, 13.41. Found: C, 57.50; H, 3.64; N, 13.36.

**Synthesis of 8-substituted-2-morpholino quinazolin-4-one (6a–e)**

***General procedure F1: alkylation with $KHCO_3$.*** Products **4a** (5.0 mmol) was reacted with $KHCO_3$ (10.0 mmol) or $K_2CO_3$ (20.0 mmol) and methyl iodide (40.0 mmol) according to previously reported procedure [23] to give **5a** and **5b**, respectively.

***General procedure F2: alkylation with $K_2CO_3$.*** Products **6a** (5.0 mmol) was reacted with $K_2CO_3$ (10.0 mmol) and methyl iodide (40.0 mmol) or benzyl bromide (40.0 mmol) according to previously reported procedure [23] to give **7a** and **7b**, respectively.

***General procedure G: reduction of nitro to amino using tin (II) chloride dihydrate.*** Products **5a** and **6a** (1.0 mmol) were reduced to the amino with $SnCl_2.2H_2O$ (6.0 mmol) according to previously reported procedure [30] to give **5c** and **6d**.

***General procedure H: reduction of nitro to amino using iron/acid in ethanol.*** Products **6b** and **7a** (2.0 mmol), iron powder (10.0 mmol), 32% hydrochloric acid (3 ml), and ethanol (20 mL) are placed in a 250 mL round bottom flask and refluxed with stirring for 90 min. The ethanol/acid is then evaporated completely under reduced pressure. 20% NaOH (50 mL) is then added to the remaining solid followed by filtration of the

resulting precipitate, which is boiled in ~100 mL of acetone then filtered. The filtrate is then evaporated to yield oil, which is triturated into a solid with ether or petroleum spirit and collected by filtration with suction to give **6e** and **7c**, respectively. The product was used without any further purification but can be purified using an appropriate technique.

***General procedure I: synthesis of 8-substituted-2-morpholino quinazolin-4-one.*** Products **5a**, **5c**, and **5d** (3.0 mmol) were reacted with morpholine for 3, 8, and 3 days, respectively, according to previously reported procedure [23] to give **6a**, **6b**, and **6c** as orange, yellow, and off-white solids, respectively. The product was used without any further purification but can be purified using an appropriate technique.

***2-(Methylthio)-8-nitroquinazolin-4(1H)-one (5a).*** White solid: Yield: 4.75 mmol (95%). Solvent: toluene. Mp 274–275°C. IR (KBr): 3388.9 (m, NH), 1666.6 (m, C═O), 1614.0 (m, NH bd), 1510.1 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 360 K): δ = 2.55 (s, 3H, H-9), 7.52 (t, $J_{6-5} = J_{6-7} = 8.0$ Hz, 1H, H-6), 8.19 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.4$ Hz, 1H, H-5), 8.27 (dd, $J_{7-6} = 8.0$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7), 12.78 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 360 K): δ = 12.4, 121.3, 124.3, 127.8, 129.6, 139.7, 144.8, 159.2, 159.2, 159.8 ppm. *Anal*. Calcd for $C_9H_7N_3O_3S$ (237.24): C, 45.57; H, 2.97; N, 17.71. Found: C, 45.60; H, 3.02; N, 17.90.

***3-Methyl-2-(methylthio)-8-nitroquinazolin-4(1H)-one (5b).*** Yield: 4.7 mmol (94%). Solvent: isopropanol. Mp 170–171°C. IR (KBr): 2931.7 (w, $sp^3$ C–H st), 1698.7 (s, C═O), 1615.5 (m, C═N), 1524.9 (s, $NO_2$), 1320.3 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 2.59 (s, 3H, H-9), 3.52 (s, 3H, H-10), 7.54 (t, $J_{6-5} = J_{6-7} = 8.0$ Hz, 1H, H-6), 8.23-8.34 (m, 2H, H-5, H-7) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 14.4, 30.0, 119.8, 124.5, 128.2, 130.4, 138.3, 144.4, 159.0, 161.3 ppm. *Anal.* Calcd for $C_{10}H_9N_3O_3S$ (251.26): C, 47.80; H, 3.61; N, 16.72. Found: C, 47.71; H, 3.66; N, 16.58.

***8-Amino-2-(methylthio)quinazolin-4(1H)-one (5c).*** White solid. Yield: 0.5 mmol (50%). Solvent: nitromethane. Mp 259–260°C. IR (KBr): 3440.1 (m, NH), 3350.0 (m, NH), 1665.7 (s, C═O), 1609.6 (m, NH bd) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 2.60 (s, 3H, H-9), 5.51 (bs, 2H, $NH_2$), 6.97 (dd, $J_{5-6} = 7.6$ Hz, $J_{5-7} = 1.6$ Hz, 1H, H-5), 7.09 (t, $J_{6-5} = J_{6-7} = 7.6$ Hz, 1H, H-6), 7.20 (dd, $J_{7-6} = 7.6$ Hz, $J_{7-5} = 1.6$ Hz, 1H, H-7), 12.23 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 12.5, 111.7, 115.8, 119.7, 125.5, 135.5, 143.2, 152.7, 161.2 ppm. *Anal.* Calcd for $C_9H_9N_3OS$ (207.25): C, 52.16; H, 4.38; N, 20.27. Found: C, 51.25; H, 4.31; N, 19.83.

***N-(2-(methylthio)-4-oxo-1,4-dihydroquinazolin-8-yl)acetamide (5d).*** Product **5c** (2.0 mmol) was refluxed in acetic anhydride (8.0 mmol) in acetic acid (15 mL) for 30 min. The reaction mixture was evaporated, triturated with petroleum spirits, and collected by filtration with suction to give compound **5d** as a white solid. Yield: 1.8 mmol (90%). Solvent: acetic acid. Mp 219–220°C. IR (KBr): 3376.2 (m, NH), 1695.3 (s, C═O), 1662.2 (s, C═O) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 2.20 (s, 3H, H-11), 2.68 (s, 3H, H-9), 7.34 (t, $J_{6-5} = J_{6-7} = 8.0$ Hz, 1H, H-6), 7.71 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.4$ Hz, 1H, H-5), 8.49 (dd, $J_{7-6} = 8.0$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7), 9.30 (bs, 1H, NH), 12.53 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 12.6, 24.0, 119.5, 119.5, 122.9, 124.9, 132.6, 138.0, 156.0, 160.5, 168.1 ppm. *Anal.* Calcd for $C_{11}H_{11}N_3O_2S$ (249.29): C, 53.00; H, 4.45; N, 16.86. Found: C, 52.77; H, 4.48; N, 16.60.

***2-Morpholino-8-nitroquinazolin-4(1H)-one (6a).*** Orange solid. Yield: 2.31 mmol (77%). Solvent: nitromethane. Mp 309–310°C (decomp.). IR (KBr): 1674.1 (s, C═O), 1528.1 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 360 K): δ = 3.67 (s, 8H, H-9, H-10), 7.20 (t, $J_{6-5} = J_{6-7} = 7.8$ Hz, 1H, H-6), 8.05 (dd, $J_{5-6} = 7.8$ Hz, $J_{5-7} = 1.6$ Hz, 1H, H-5), 8.14 (dd, $J_{7-6} = 7.8$ Hz, $J_{7-5} = 1.6$ Hz, 1H, H-7), 11.38 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 360 K): δ = 44.7, 65.3, 118.7, 120.2, 128.3, 129.8, 142.6, 143.9, 151.5, 161.5 ppm. *Anal*. Calcd for $C_{12}H_{12}N_4O_4$ (276.25): C, 52.17; H, 4.38; N, 20.28. Found: C, 52.03; H, 4.41; N, 20.31.

***3-Methyl-2-morpholino-8-nitroquinazolin-4(3H)-one (6b).*** Yellow solid. Yield: 2.31 mmol (77%). Solvent: isopropanol. Mp 309–310°C (decomp.). IR (KBr): 2926.6 (w, $sp^3$ C–H st), 2860.1 (w, $sp^3$ C–H st), 1683.3 (s, C═O), 1524.2 (m, $NO_2$), 1360.3 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 3.31 (t, $J_{10-9} = 4.8$ Hz, 4H, H-10), 3.48 (s, 3H, H-11), 3.74 (t, $J_{9-10} = 4.8$ Hz, 4H, H-9), 7.41 (t, $J_{6-5} = J_{6-7} = 7.8$ Hz, 1H, H-6), 8.16 (dd, $J_{7-6} = 7.8$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7), 8.25 (dd, $J_{5-6} = 7.8$ Hz, $J_{5-7} = 1.4$ Hz, 1H, H-5) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 33.1, 48.9, 65.1, 119.9, 122.7, 128.0, 130.0, 139.5, 144.6, 156.0, 161.8 ppm. *Anal*. Calcd for $C_{13}H_{14}N_4O_4$ (290.27): C, 53.79; H, 4.86; N, 19.30. Found: C, 53.61; H, 4.83; N, 19.20.

***N-(2-morpholino-4-oxo-1,4-dihydroquinazolin-8-yl)acetamide (6c).*** Off-white solid. Yield: 2.9 mmol (58%). Solvent: nitromethane. Mp 320–340°C (decomp.) IR (KBr): 3367.2 (m, NH), 2956.5 (w, $sp^3$ C–H st.), 1661.7 (s, C═O) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 360 K): δ = 2.19 (s, 3H, H-12), 3.70 (s, 8H, H-9, H-10), 7.09 (t, $J_{6-5} = J_{6-7} = 7.8$ Hz, 1H, H-6), 7.61 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.4$ Hz, 1H, H-5), 8.43 (dd, $J_{7-6} = 7.8$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7), 9.10 (bs, 1H, NH), 11.33 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 360 K): δ = 24.1, 44.9, 65.5, 116.4, 119.3, 121.5, 122.0, 131.7, 139.7, 150.2, 162.4, 167.9 ppm. *Anal.* Calcd for $C_{14}H_{16}N_4O_3$ (288.30): C, 58.32; H, 5.59; N, 19.43. Found: C, 58.38; H, 5.58; N, 19.52.

***8-Amino-2-morpholinoquinazolin-4(1H)-one (6d).*** Light-yellow solid. Yield: 3.0 mmol (60%). $R_f = 0.62$ (methanol–ethylacetate, 5:95). Mp 229–230°C IR (KBr): 3440.7 (m, NH), 3342.2 (m, NH), 2959.8 (w, $sp^3$ C–H st), 1673.6 (s, C═O), 1614.0 (s, NH bd.) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 3.59 (t, $J_{9-10} = 4.6$ Hz, 4H, H-9), 3.69 (t, $J_{10-9} = 4.6$ Hz, 4H, H-10), 5.23 (bs, 2H, $NH_2$), 6.81–6.93 (m, 2H, H-5, H-6), 7.14 (dd, 1H, H-7, $J_{7-6} = 7.0$ Hz, $J_{7-5} = 2.2$ Hz), 11.10 (bs, 1H, NH) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 45.2, 65.4, 111.9, 114.8, 116.8, 122.5, 137.4, 142.3, 149.3, 163.0 ppm. *Anal*. Calcd for $C_{12}H_{14}N_4O_2$ (246.27): C, 58.53; H, 5.73; N, 22.75. Found: C, 58.32; H, 5.74; N, 22.80.

***8-Amino-3-methyl-2-morpholinoquinazolin-4(3H)-one (6e).*** Off-white solid. Yield: 1.42 mmol (71%). $R_f = 0.57$ (2-propanol-chloroform, 5:95). Mp 126–127°C. IR (KBr): 3457.6 (m, NH), 3326.6 (m, NH), 2857.0 (w, $sp^3$ C–H st), 1675.0 (m, C═O), 1614.3 (s, NH bd) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 340 K): δ = 3.19 (t, $J_{9-10} = 4.6$ Hz, 4H, H-9), 3.48 (s, 3H, H-11), 3.77 (t, $J_{10-9} = 4.6$ Hz, 4H, H-10), 5.40 (bs, 2H, NH), 6.92 (dd, 1H, H-7, $J_{7-6} = 7.8$ Hz, $J_{7-5} = 1.6$ Hz), 7.05 (t, 1H, H-6, $J_{6-5} = J_{6-7} = 7.8$ Hz), 7.21 (dd, $J_{5-6} = 7.8$ Hz, $J_{5-7} = 1.6$ Hz, 1H, H-5) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 31.6, 49.5, 65.4, 112.0, 114.9, 118.4, 124.8, 134.1, 143.1, 152.9, 163.0 ppm. *Anal*. Calcd for $C_{13}H_{16}N_4O_2$ (260.29): C, 59.99; H, 6.20; N, 21.52. Found: C, 60.05; H, 6.28; N, 21.56.

***4-(4-Methoxy-8-nitroquinazolin-2-yl)morpholine (7a).*** Orange solid. Yield: 3.9 mmol (78%). Solvent: methanol. Mp 142–143°C IR (KBr): 2860.1 (w, $sp^3$ C–H st), 1636.1 (m, C═N), 1531.5 (s, $NO_2$) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, T = 300 K): δ = 3.77 (t, $J_{9-10} = 5.0$ Hz, 4H, H-9), 3.95 (t, $J_{10-9} = 5.0$ Hz, 4H, H-10), 4.10

(s, 3H, H-11), 7.11 (t, $J_{6-5} = J_{6-7} = 8.0$ Hz, 1H, H-6), 8.03 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.6$ Hz, 1H, H-5), 8.10 (dd, $J_{7-6} = 8.0$ Hz, $J_{7-5} = 1.6$ Hz, 1H, H-7) ppm. $^{13}$C-NMR ($CDCl_3$, T = 300 K): δ = 44.4, 54.3, 66.9, 113.4, 119.4, 128.8, 128.9, 144.0, 145.9, 158.9, 167.2 ppm. *Anal*. Calcd for $C_{13}H_{14}N_4O_4$ (290.27): C, 53.79; H, 4.86; N, 19.30. Found: C, 53.84; H, 4.93; N, 19.34.

***4-(4-(Benzyloxy)-8-nitroquinazolin-2-yl)morpholine (7b).*** Orange solid. Yield: 3.6 mmol (72%). Solvent: methanol. Mp 142–143°C. IR (KBr): 2860.0 (w, $sp^3$ C–H st), 1630.1 (s, C═N), 1522.5 (s, $NO_2$), 1356.0 (m, $NO_2$) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, T = 300 K): δ = 3.76 (t, $J_{9-10} = 4.8$ Hz, 4H, H-9), 3.94 (t, $J_{10-9} = 4.8$ Hz, 3H, H-10), 5.55 (s, 2H, H-11), 7.10 (t, $J_{6-5} = J_{6-7} = 8.0$ Hz, 1H, H-6), 7.35–7.49 (m, 5H, benzylic hydrogens), 8.02 (dd, $J_{5-6} = 8.0$ Hz, $J_{5-7} = 1.4$ Hz, 1H, H-5), 8.15 (dd, $J_{7-6} = 8.0$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7) ppm. $^{13}$C-NMR ($CDCl_3$, T = 300 K): δ = 44.5, 66.9, 68.8, 113.4, 119.5, 128.1, 128.5, 128.7, 128.9, 129.0, 135.8, 144.0, 146.1, 158.8, 166.5 ppm. *Anal*. Calcd for $C_{19}H_{18}N_4O_4$ (266.37): C, 62.29; H, 4.95; N, 15.29. Found: C, 61.68; H, 4.93; N, 14.81.

***4-Methoxy-2-morpholinoquinazolin-8-amine (7c).*** White solid. Yield: 0.8 mmol (40%). $R_f = 0.72$ (ethylacetate-petroleum spirit, 3:2). Mp 120–121°C. IR (KBr): 3410.8 (m, NH), 3303.9 (m, NH), 2860.9 (w, $sp^3$ C–H st), 1623.2 (m, NH bd) $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$, T = 380 K): δ = 3.72 (t, $J_{9-10} = 4.8$ Hz, 4H, H-9), 3.83 (t, $J_{10-9} = 4.8$ Hz, 4H, H-10), 4.07 (s, 3H, H-11), 5.27 (bs, 2H, $NH_2$), 6.86 (dd, $J_{7-6} = 7.4$ Hz, $J_{7-5} = 1.4$ Hz, 1H, H-7), 6.94 (t, $J_{6-5} = J_{6-7} = 7.4$ Hz, 1H, H-6), 7.10 (dd, 1H, H-5, $J_{5-6} = 7.4$ Hz, $J_{5-7} = 1.4$ Hz) ppm. $^{13}$C-NMR (DMSO-$d_6$, T = 340 K): δ = 44.1, 53.3, 65.8, 109.0, 110.3, 112.4, 122.2, 141.7, 141.9, 156.2, 166.7 ppm. *Anal*. Calcd for $C_{13}H_{16}N_4O_2$ (260.29): C, 59.99; H, 6.20; N, 21.52. C, 60.25; H, 6.17; N, 21.78.

## ANTIBACTERIAL ASSAY

The disk diffusion method with Mueller–Hinton agar was used to evaluate the antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. faecalis*. All bacteria were grown from frozen cultures in nutrient broth by overnight incubation, diluted to give an $OD_{600}$ of 0.95–1.05 then evenly applied to the appropriately labeled agar plates using a sterile swab. Blank antibacterial susceptibility disks (three per plate) were then placed on each agar plate. A total of 2.5 μL of each compound as a 20 mM solution in DMSO (50 nmol) was applied to the appropriate blank antibacterial susceptibility disks for each bacterial strain. Ciprofloxacin and Penicillin G potassium salt, of the same concentration, were used as positive controls and a DMSO blank as a negative control. The diameter of the zone of inhibition was measured in millimeters (mm) after 24 h of incubation at 37°C.

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