Synthesis, Antiplasmodial and Antitrypanosomal Evaluation of a Series of Novel 2-Oxoquinoline-based Thiosemicarbazone Derivatives

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ABSTRACT

Herein a series of novel thiosemicarbazones (TSCs) derived from 2-oxoquinoline scaffold is reported, and the target compounds have been successfully synthesized and characterized using standard spectroscopic techniques. The *in vitro* biological activities of synthesized molecules were evaluated against *Plasmodium falciparum* malaria parasites (strain 3D7), *Trypanosoma brucei brucei* parasites (strain 427) and HeLa cells. All the compounds displayed modest or no activity at a concentration of 20 μM and percentage viability of >50 % was often observed. Except for compound 9o, none of the final compounds exhibited cytotoxic effects against HeLa cells at 20 μM.

KEYWORDS

***Trypanosoma brucei*, trypanosomiasis, *Plasmodium falciparum*, thiosemicarbazones, 2-oxoquinoline.**

1. Introduction

Malaria, an infectious parasitic disease, is a major health risk in many developing countries worldwide.[1] Despite tremendous progress over the last two decades, in 2017 there were 216 million cases of malaria infection, with an estimated 445 000 deaths, 90 % of which occur in sub-Saharan Africa.[1,2] Currently, it is estimated that almost 3.2 billion people globally are at risk of contracting the disease.[3] This is further aggravated by the widespread drug and multidrug resistant *Plasmodium falciparum* parasite, the main cause of infection in humans, to almost all antimalarial drugs that are in clinical use.[4] In the absence of an effective malaria vaccine, the need to discover and develop new antimalarial drugs, with unique structural motifs and new mode of action, that are safe and effective against highly resistant parasites is imperative.[4,5]

On other hand, human African trypanosomiasis (HAT), commonly referred to as a sleeping sickness, is caused by protozoan parasites of the genus *Trypanosoma*, and the two species that are transmitted to humans by blood-feeding tsetse flies (*Glossina spp.*) are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.[6,7] Up to 70 million people, in various parts of the 36 countries in Africa where the disease is endemic, are at risk of infection.[6] While the cases of HAT in Africa have been reasonably low, in 2015 an estimated 3000 new infections of East and West African trypanosomiasis were reported to the World Health Organisation (WHO).[8] Regrettably, in pregnant women or those of child-bearing age, the disease causes infertility and abortion, and it is invariably fatal if left untreated.[8] Currently, only a handful of drugs are available for the treatment of HAT, and are utilized based on the causative trypanosome species and stage of the disease.[8] For example, pentamidine and suramin are recommended for treatment of the acute initial stage of *T. b. gambiense*, while a combination of nifurtimox-eflornithine and melarsoprol are deployed for the secondary stage of the disease. For *T. b. rhodesiense*, suramin is a preferred drug for treatment of the initial stage of the disease, while melarsoprol is reserved for secondary stage chemotherapy.[9] However; these drugs have shortcomings and some of them are associated with life-threatening side effects that have prompted the scientific community to search for new compounds with desirable safety margins and drug-like properties to replace them.

Thiosemicarbazones are a class of compounds which have enjoyed significant attention due to their broad-spectrum of biological activities, including antibacterial, antiprotozoal, antifungal, antiviral and antitumour activity.[10] Quinoline and related derivatives, on the other hand, are useful compounds with diverse pharmaceutical applications, and some have even reached markets for treatment of various ailments.[11] The 2-oxoquinoline (2-OCQ), which belongs to a quinoline family, is an interesting naturally occurring scaffold to attach new moieties or bioactive groups and it has been widely used as a 'parental' framework to synthesize a variety of molecules with a wide range of biological activities such as antitubercular, anti-inflammatory, antifungal and antileishmanial activity.[12–15] For example, oxoquinoline-derived thiosemicarbazones **I** and **III** (Fig. 1) were found to exhibit good antiproliferative activity against the HCT116 cell line.[16]

In our pursuit of developing biologically relevant molecules, that could address some of the problems associated with

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I II

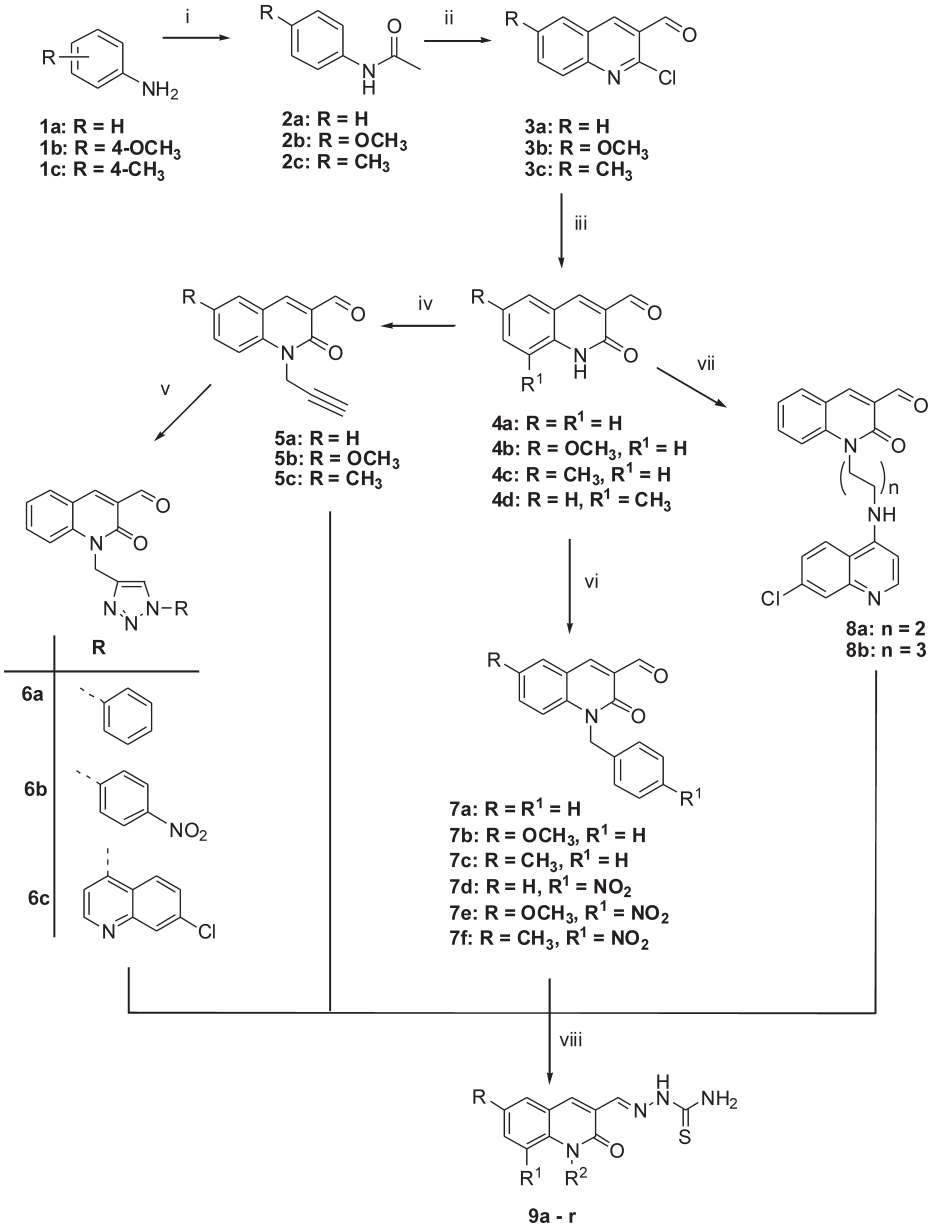
Figure 1 Chemical structures of biologically active quinoline-derived thiosemicarbazone derivatives (**I** and **II**) based on 2-oxo-quinoline structural motif.

infections caused by protozoan parasites, we are interested in exploring a class of 2-oxoquinoline-derived thiosemicarbazone derivatives as antiplasmodial and antitrypanosomal agents. To the best of our knowledge, there has been no report on the antiplasmodial and antitrypanosomal properties of these compounds in literature. To this end, in this study we report on the synthesis of quinoline-derived thiosemicarbazones and their *in vitro* bioassay screening against *P. falciparum* 3D7 strain and *T. b. brucei* (strain 427) as well as cytotoxicity evaluation against HeLa cell lines.

2. Results and Discussion

2.1. Synthesis

The synthesis of target quinoline-derived thiosemicarbazone derivatives is outlined in Scheme 1. Commercially available



Scheme 1

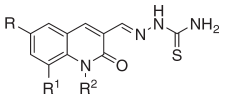
(i) Acetic acid, acetic anhydride (1:1), reflux, 0.5 h; (ii) DMF, $POCl_3$, 85 °C, 12 h; (iii) acetic acid, 90 °C, 8 h; (iv) propargyl bromide, DMF, K_2CO_3, 25 –60 °C, 4 h; (v) azide, CuI, 2,6-lutidine, 0 °C, 12 h; (vi) benzylbromides (R^3-X), DMF, K_2CO_3, r.t – 60 °C, 1–12 h; (vii) aminoquinolines, DMF, 0 °C, NaH, 1 h, 60 °C, 12 h; (viii) thiosemicarbazide, MeOH or EtOH, 80 °C, 10 –15 h.

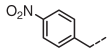
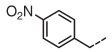
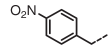
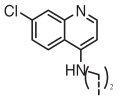
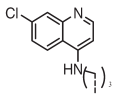
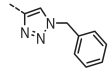
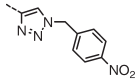
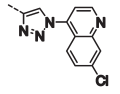
anilines **1a–c** were treated with a solution of acetic acid and acetic anhydride (1:1 mole ratio) under reflux to generate the corresponding acetanilides **2a–c** in moderate yields.[17] The Vilsmeier-Haack reaction[18] involving the condensation of resultant acetanilides **2a–c** with *N*,*N*-dimethylformamide (DMF), in the presence of phosphorusoxychloride ($POCl_3$), was then used to produce 2-chloroquinoline-3-carbaldehyde derivatives **3a–c**.[18]

The next step involved accessing the desired key intermediates, 2-oxoquinoline-3-carbaldehyde derivatives **4a–d**, *via* a previously reported literature methods.[19] Thus, the hydrolytic reaction of **3a–c** in 70 % acetic acid aqueous solution resulted in quinolinone derivatives **4a–c**, which were obtained in yields ranging from 38–62 %. To access compounds **4d**, commercial available 2-chloroquinoline-3-carbaldehyde was reacted, under similar reaction conditions as in the synthesis of **4a–c**, to yield the desired compound **4d** in 69 % yield. With the desired key intermediates (**4a–c**) in hand, propargylation reaction using propargyl bromide yielded propargyl quinoline aldehydes **5a–c**, which were then further reacted with appropriate azides, under the copper-catalyzed azide-alkyne cycloaddition (CuAAC) conditions,[20–23] to form 1,4-disubstituted-1,2,3-triazoles quinoline aldehydes **6a–c** in yields ranging from 42–61 %.

Similarly, reacting the key intermediates **4a–c** with substituted benzylbromides and 7-chloro-*N*-(2-chloropropyl)quinolin-4-amine yielded quinoline aldehydes **7a–f** and **8b** in moderate yields, respectively. Compound **8a** could not be isolated in its pure form, and instead it was reacted with thiosemicarbazide (step viii) as crude product to form the desired quinoline-derived thiosemicarbazone **9n**. Lastly, all the quinoline aldehydes **5a–c**, **6a–c**, **7a–f** and **8b** were then subjected to the Schiff base condensation reaction with commercially accessible thiosemicarbazide in refluxing MeOH or EtOH to give rise to the desired 2-oxoquinoline-based thiosemicarbazone derivatives **9a–r** (Table 1) in moderate to excellent yields.[24–26] All the intermediates and target compounds were fully characterized by analytical and spectroscopic techniques.

Table 1 Synthesized quinoline-based thiosemicarbazones 9a–r and their isolated yields.



Comp	R	R^1	R^2	Yield/%
9a	H	H	H	70
9b	H	CH_3	H	68
9c	CH_3	H	H	60
9d	OCH_3	H	H	63
9e	H	H		42
9f	H	H		54
9g	OCH_3	H		38
9h	OCH_3	H		44
9i	CH_3	H		41
9j	CH_3	H		57
9k	H	H		69
9l	CH_3	H		56
9m	OCH_3	H		69
9n	H	H		56
9o	H	H		48
9p	H	H		45
9q	H	H		55
9r	H	H		42

2.2. *In Vitro* Biological Evaluation

The prepared target compounds were evaluated *in vitro* for antiplasmodial activity against the chloroquine sensitive (CQS) 3D7 *P. falciparum*, the trypanosomal subspecies responsible for nagana *T. b. brucei*, and for cytotoxicity evaluation using a human cervix adenocarcinoma (HeLa) cell line. Chloroquine (CQ) was included as a positive control for *P. falciparum* and pentamidine (PMD) was employed for *T. b. brucei* assays while emetine (EMT) was a positive control drug for HeLa cells. The screening assay was done using the malaria parasite lactate dehydrogenase *Pf*(pLDH), *T. b. brucei* and HeLa cell resazurin assays that were performed in duplicates using 20 mM final concentrations of each compound. The percentage cell viability results upon exposure of *P. falciparum*, *T. b. brucei* and HeLa cells to the compounds are displayed in Table 2.

The tested compounds (Table 2) exhibited no cytotoxic effects (percentage viability >64 %) as measured by the viability of HeLa cell lines at a concentration of 20 μM, the exception is compound **9o** which reduced HeLa cell viability to 6.6 %. Excluding compounds **9m**, **9n** and **9o**, which reduced the percentage parasite viability to below 20 %, none of the tested target compounds displayed desirable potency at the concentration of 20 μM. These compounds were evaluated further to determine the corresponding IC_{50} values (Fig. 2) against the 3D7 strain of the parasite *P. falciparum*. And while **9m** emerged as inactive, compounds **9n** and **9o** displayed notable activity with the corresponding IC_{50} values of 2.09 and 1.79 μM, respectively. These data suggest that the observed antiplasmodial activity may be related to the presence of aminoquinoline moiety in each molecule, which is known to bind with haem, thus preventing the formation of haemozoin. However, the corresponding quinoline aldehyde intermediates **6c** and **8b** were ineffective (data not included) at the maximum tested concentration

Table 2: *In vitro* antiplasmodial and antitrypanosomal activity, and cytotoxicity evaluation of target compounds **9a–r**. The IC_{50} values (in μM) obtained with the standard drug compounds CQ, PMD and EMT are also shown.

	% Viability at 20 μM		
Comp	3D7	*T. brucei*	Cytotoxicity
9a	79.1	114.5	84.8
9b	66.1	103.1	64.8
9c	78.5	115.1	110.2
9d	81.6	112.3	95.1
9e	97.1	116.1	89.9
9f	49.3	104.3	85.1
9g	97.1	52.8	102.3
9h	81.6	118.4	83.3
9i	72.2	108.6	96.0
9j	101.4	102.3	94.1
9k	103.9	92.7	62.0
9l	85.9	111.7	111.2
9m	**17.6**	90.3	100.2
9n	**–2.5**	**12.7**	90.8
9o	**–4.4**	**12.8**	**6.6**
9p	87.0	115.3	112.4
9q	74.5	98.8	106.8
9r	31.3	112.8	105.5
CQ	**0.028**	–	–
PMD	–	**0.0028**	–
EMT	–	–	**0.37**

(20 mM) suggesting that enhanced activity of **9n** and **9o** could be attributed to the contribution of the thiosemicarbazone and 4-aminoquinoline fragments.[27]

Similarly, in terms of antitrypanosomal activity, none of the tested compounds exhibited appreciable activity except compounds **9n** and **9o**, which reduced the viability of trypanosomes (*T. b. brucei*) at 20 μM to 12.7 % and 12.8 %, respectively. Since compound **9o** showed a significant cytotoxic effect at 20 μM, only compound **9n** was further screened to determine the corresponding IC_{50} value (Fig. 3). Despite significant growth inhibition as measured by the viability of trypanosomes, compound **9n** was found to be inactive with IC_{50} value of 167.7 μM.

3. Conclusion

In summary, a series of thiosemicarbazone derivatives **9a–r** based on the 2-oxoquinoline structural motif have been prepared in moderate to excellent yields and their structural integrity confirmed using various spectroscopic techniques. Despite the poor antiplasmodial and antitrypanosomal activity of the majority of the tested compounds, the promising potency of **9n** and **9o** provides an avenue for further in-depth investigation of these bi-quinoline thiosemicarbazone compounds as a new family of quinoline-based anti-infective agents. Apart from compounds **9m**, **9n** and **9o**, which exhibited weak to good activity with IC_{50} (*T. b. brucei*) = 167.7 μM and IC_{50}s (*Pf*) = 2.09 and 1.79 μM values, the rest of compounds were inactive and parasite percentage viabilities of >50 % were often observed. As determined by the HeLa cell line, the majority of these compounds showed no significant toxicity.

4. Experimental

4.1. General

All commercially available chemicals and reagents were purchased from Sigma-Aldrich (Pty) Ltd and Merck (Pty) Ltd, and were used without further purification unless stated otherwise. The progress of reactions were monitored by analytical thin layer chromatography (TLC) using Merck F_{254} silica gel plates (supported on aluminium), which were visualized under ultraviolet (UV 254 and 366 nm) light or, where necessary, stained in iodine flask. The crude compounds were purified by flash column chromatography using Merck Kieselgel 60 Å: 70–230 silica gel mesh or by preparative thin-layer chromatography (PTLC) using Merck 60GF_{254} silica gel coated on glass plates (2.0 × 200 × 200 mm). The ^{1}H and ^{13}CNMR spectra were recorded on either a Bruker Fourier 300 or a 400 MHz spectrometer. Spectra were recorded in deuterated solvents: $CDCl_3$-d_6 and DMSO-d_6. All chemical shift values are reported in parts per million (ppm) referenced to residual solvent resonances ($CDCl_3$ δ_H 7.26, δ_C 77.2; DMSO δ_H 2.50, δ_C 39.5). The coupling constants are given in Hertz. High resolution mass spectrometry was performed on a Waters Synapt G2 TOF instrument with an ESI source, University of Stellenbosch. Measurement of the melting points was carried out using a Reichert hot stage microscope (Protea Holdings Ltd.) and uncorrected. Elemental microanalysis was per-

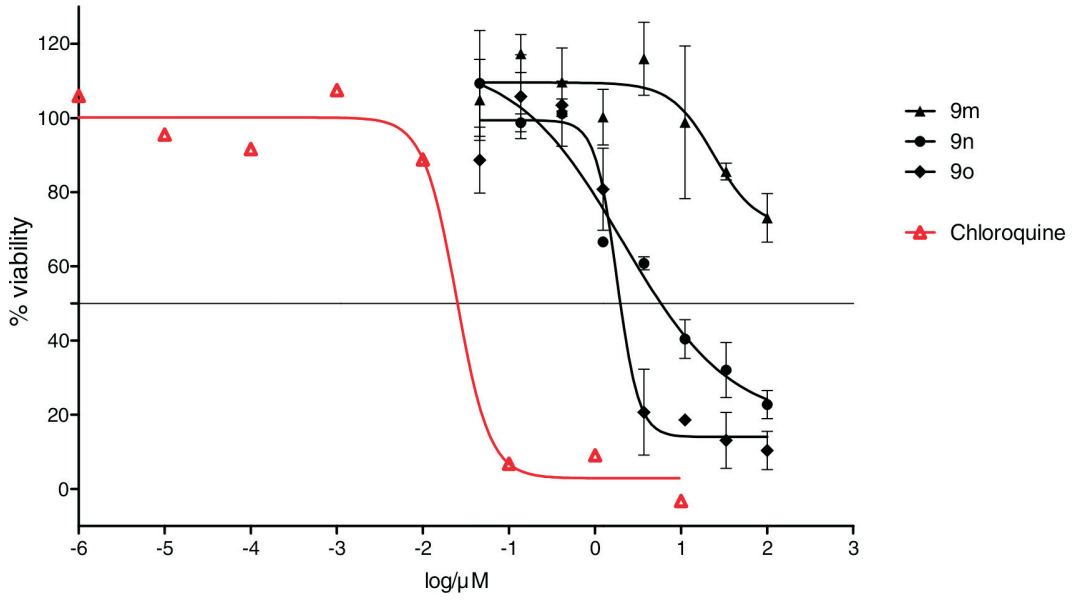


Figure 2 Plot of percentage antiplasmodial activity against log concentration for compounds **9m**, **9n**, **9o** and the standard drug, chloroquine.

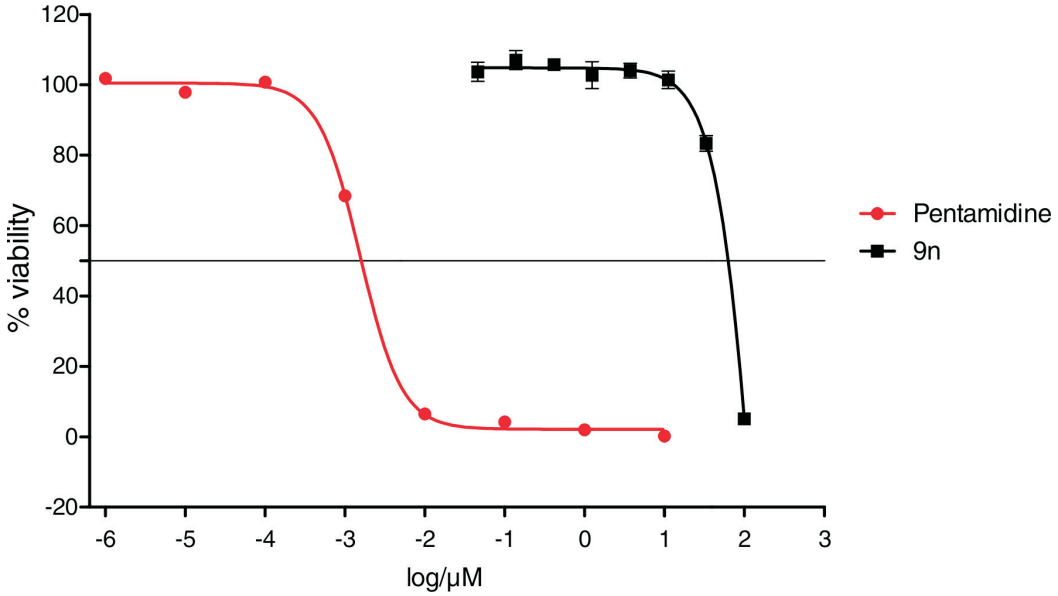


Figure 3 Plot of percentage antitrypanosomal activity against log concentration for compound **9n** and the standard drug, pentamidine.

formed on Elementar Analysensysteme varioMICRO V1.6.2 GmbH analysis system.

4.2. General Procedure for Synthesis of Thiosemicarbazone Compounds 9a–r

An appropriate starting 2-oxoquinoline-3-carbaldehyde (0.081 g, 0.5), and thiosemicarbazide (0.5 mmol) were mixed in methanol (25 mL) and heated to 80 °C for 10 h. After reaction completion, the resulting product was allowed to cool to ambient temperature and resulted in the formation of a precipitate, which was filtered, washed with ice-cold MeOH and dried to give the desired products.

(E)-2-((2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9a**): 70 % yield; yellow solid; mp 290–293 °C (Lit[28] 296 °C); δ_H(300 MHz; DMSO-d_6): 12.1 (1H, s, N(1)H), 11.7 (1H, s, N(11)H), 8.83 (1H, s, H_4), 8.76 (1H, s, H_9), 8.32 (1H, br s, N*H*H), 8.27 (1H, s, H_4), 8.11 (1H, br s, NH*H*), 7.64 (1H, dd, J = 1.1 and 8.0 Hz, H_5), 7.52 (1H, ddd, J = 1.4, 7.3 and 9.2 Hz, H_6), 7.30 (1H, dd, J = 0.8 and 8.0 Hz, H_8), 7.22 (1H, ddd, J = 0.9, 7.3 and 9.1 Hz, H_7) ppm; δ_C (75 MHz, DMSO-d_6): 178.1, 161.0, 139.0, 136.8, 135.2, 131.1, 128.6, 125.4, 122.5, 119.3, 115.3 ppm; υ_{max}(neat, cm^{-1}): 3291 (NH), 3173 (NH), 1638 (C=O), 1533 (C=S), 851 (C-S); HRMS (ESI) m/z calcd for $C_{11}H_{10}N_4OS$ 246.0575, found 247.0662 $[M+H]^+$; Anal. calcd for $C_{11}H_{10}N_4OS{\cdot}0.125H_2O$: C, 53.16; H, 4.16; N, 22.54; S, 12.90 %. Found: C, 53.18; H, 3.95; N, 22.54; S, 12.96 %.

(E)-2-((8-Methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9b**): 63 % yield; yellow solid; mp 246–248 °C; δ_H(300 MHz; DMSO-d_6): 11.6 (1H, s, N(1)H), 11.2 (1H, s, N(11)H), 8.76 (1H, s, H_9), 8.29 (2H, s, N*H*H), 8.28 (1H, s, H_4), 8.01 (1H, br s, NH*H*), 7.50 (1H, d, J = 7.8 Hz, H_5), 7.37 (1H, d, J = 7.5 Hz, H_7), 7.14 (1H, t, J = 8.0 Hz, H_6), 2.43 (3H, s, CH_3) ppm; δ_C(75 MHz, DMSO-d_6): δ_C 178.1, 161.5, 137.3, 136.7, 135.8, 132.3, 126.7, 125.0, 123.6, 122.2, 119.3, 17.2 ppm; υ_{max}(neat, cm^{-1}): 3269 (NH), 3154 (NH), 1645 (C=O), 1531 (C=S), 856 (C-S); HRMS (ESI) m/z calcd for $C_{12}H_{12}N_4OS$: 260.0732, found 261.0808 $[M+H]^+$; Anal. calcd for $C_{12}H_{12}N_4OS{\cdot}0.75H_2O$: C, 52.64; H, 4.97; N, 20.46; S, 11.71 %. Found: C, 52.61; H, 4.97; N, 20.48; S, 11.57 %.

(E)-2-((6-Methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide, (**9c**): 60 % yield; orange solid; mp 248–250 °C; δ_H(300 MHz; DMSO-d_6): 11.9 (1H, s, N(1)H), 11.6 (1H, s, N(11)H), 8.68 (1H, s, H_9), 8.29 (1H, br s, NH*H*), 8.27 (1H, s, H_4), 8.07 (1H, s, N*H*H), 7.27 (3H, m, H_5, H_7 and H_8) 2.33 (3H, s, CH_3) ppm; δ_C(75 MHz, DMSO-d_6): δ 178.1, 160.9, 137.0, 136.9, 134.7, 132.3, 131.3, 127.9, 125.2, 119.2, 115.1, 20.4 ppm; υ_{max}(neat, cm^{-1}): 3256 (NH), 3148 (NH), 1525 (C=N), 1646 (C=N), 1208 (C=S), 863 (C-S); HRMS (ESI) m/z calcd for $C_{12}H_{12}N_4OS$ $[M+H]^+$ 260.0732, found 261.0823; Anal. calcd for $C_{12}H_{12}N_4OS{\cdot}0.75H_2O$: C, 52.64; H, 4.97; N, 20.46; S,11.71 %. Found: C, 52.51; H, 5.08; N, 20.13; S, 11.50 %.

(E)-2-((6-Methoxy-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9d**): 69 % yield; orange solid; mp 255–256 °C (Lit.[29] 258–260 °C); δ_H(300 MHz; DMSO-d_6): 11.8 (1H, s, N(1)H), 11.6 (1H, s, N(11)H), 8.72 (1H, s, H_9), 8.31 (1H, br s, N*H*H), 8.27 (1H, s, H_4), 8.05 (1H, br s, NH*H*), 7.26 (1H, d, J = 8.9 Hz H_8), 7.18 (1H, dd, J = 2.8 and 8.9 Hz, H_7), 7.12 (1H, d, J = 2.8 Hz, H_5), 3.71 (3H, s, CH_3) ppm; δ_C(75 MHz, DMSO-d_6): 178.1, 160.5, 154.4, 136.8, 134.7, 133.6, 125.6, 120.5, 119.7, 116.5, 109.2, 55.4 ppm; υ_{max}(neat, cm^{-1}): 3392 (NH), 3160 (NH), 1647 (C=O), 1530 (C=S), 840 (C-S); HRMS (ESI) m/z calcd for $C_{12}H_{12}N_4O_2S$: 276.0681, found 277.0747 $[M+H]^+$. Anal. Calcd for $C_{12}H_{12}N_4O_2S$: C, 52.16; H, 4.38; N, 20.28; S, 11.60. Found (%): C, 52.11; H, 4.28; N, 20.23; S, 11.57 %.

(E)-2-((1-Benzyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9e**): 42 % yield; yellow solid; mp 222–224 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.86 (1H, s, H_9), 8.39 (1H, s, H_4), 8.34 (1H, br s, N*H*H), 8.14 (1H, br s, NH*H*), 7.73 (1H, dd, J = 1.2 and 7.8 Hz, H_5), 7.53 (1H, ddd, J = 1.4, 7.5 and 8.6 Hz, H_6), 7.43 –7.18 (7H, m, Ar-Hs), 5.57 (2H, s, H_{1a}) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.6, 138.9, 137.0, 135.7, 134.7, 131.3, 129.6, 128.6, 127.1, 126.5, 124.5, 122.7, 120.2, 115.2, 45.0 ppm; υ_{max}(neat, cm^{-1}): 3201 (NH), 3147 (NH), 1626 (C=O), 1517 (C=N), 1207 (C=S), 855 (C-S); HRMS (ESI) m/z calcd for $C_{18}H_{16}N_4OS$ 336.1045, found 337.1136 $[M+H]^+$; Anal. calcd for $C_{18}H_{16}N_4OS$: C, 64.26; H, 4.79; N, 16.65; S, 9.53. Found: C, 65.16; H, 5.86; N, 14.99; S, 8.63 %.

(E)-2-((1-(4-Nitrobenzyl)-2-oxo-1,2-dihydroquinolin-3-ylyl)methylene)hydrazinecarbothioamide (**9f**): 52 % yield; yellow solid; mp 235–237 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.89 (1H, s, H_9), 8.38 (1H, s, H_4), 8.36 (1H, br s, N*H*H), 8.20–8.16 (2H, m, $H_{3'}$ and $H_{5'}$), 8.15 (1H, br s, NH*H*), 7.76 (1H, d, J = 7.6 Hz, H_8), 7.54

(1H, t, J = 8.7 Hz, H_6), 7.37 (1H, d, J = 8.7 Hz, H_5), 7.31 (2H, t, 7.4 Hz, H_7), 5.70 (2H, s, H_{1a}) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.6, 146.6, 144.6, 138.7, 136.8, 135.0, 131.5, 129.8, 127.8, 124.6, 123.8, 123.0, 120.3, 115.3, 44.9 ppm; v_{max}(neat, cm^{-1}): 3216 (NH), 3148 (NH), 1633 (C=O), 1516 (C=N), 1203 (C=S), 850 (C-S); HRMS (ESI) *m/z* calcd for $C_{18}H_{13}N_5O_3S$ 381.0896, found 382.0968 $[M+H]^+$; Anal. calcd for $C_{18}H_{13}N_5O_3S{\cdot}0.5CH_3OH$: C, 56.19; H, 3.82; N, 17.71; S, 8.11 %. Found: C, 56.61; H, 3.91; N, 17.38; S, 8.34 %.

(E)-2-((1-Benzyl-6-methoxy-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9g**): 41 % yield; yellow solid; mp 232–234 °C, δ_H(400 MHz; DMSO-d_6): δ 11.7 (1H, s, N(11)H), 8.81 (1H, s, H_9), 8.38 (1H, s, H_4), 8.36 (1H, br s, N*H*H), 8.09 (1H, br s, NH*H*), 7.36–7.27 (3H, m, H_5, H_7 and H_8), 7.25–7.15 (5H, m, $H_{2'}$,$H_{3'}$,$H_{4'}$,$H_{5'}$ and $H_{6'}$), 5.57 (2H, s, H_{1a}), 3.79 (3H, s, CH_3) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.1, 154.4, 137.1, 136.6, 134.3, 133.5, 128.6, 127.0, 126.5, 124.9, 120.9, 120.0, 116.6, 110.8, 55.4, 45.0 ppm; v_{max}(neat, cm^{-1}): 3275 (NH), 3157 (NH), 1635 (C=O), 1518 (C=N), 1202 (C=S), 869 (C-S); HRMS (ESI) *m/z* calcd for $C_{19}H_{18}N_4O_2S$: 366.1150, found 367.1213 $[M+H]^+$; Anal. calcd for $C_{19}H_{18}N_4O_2S{\cdot}0.25H_2O$; C, 61.52; H, 5.03; N, 15.10; S, 8.64 %. Found: C, 61.37; H, 4.92; N, 15.03; S, 8.72 %.

(E)-2-((6-Methoxy-1-(4-nitrobenzyl)-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9h**): 57 % yield; yellow solid; mp 238–240 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.85 (1H, s, H_4), 8.37 (1H, br s, N*H*H), 8.17 (2H, d, J = 8.8 Hz, $H_{3'}$and $H_{5'}$), 8.10 (1H, br s, NH*H*), 7.45 (2H, d, J = 8.8 Hz, $H_{2'}$ and $H_{6'}$), 7.31 (1H, d, J = 9.3 Hz, H_8), 7.24 (1H, d, J = 2.9 Hz, H_5), 7.17 (1H, dd, J = 2.9 and 9.2 Hz, H_7), 5.68 (2H, s, H_{1a}), 3.80 (3H, s, CH_3) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.1, 154.6, 146.6, 144.7, 136.9, 134.5, 133.3, 127.7, 124.9, 123.8, 121.0, 120.1, 116.4, 111.8, 55.4, 44.8 ppm; v_{max}(neat, cm^{-1}): 3215 (NH), 3155 (NH), 1636 (C=O), 1511 (C=N), 1206 (C=S), 860 (C-S); HRMS (ESI) *m/z* calcd for $C_{19}H_{17}N_5O_4S$: 411.1001, found 412.0810 $[M+H]^+$; Anal. calcd for $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02; S, 7.79 %. Found: C, 55.32; H, 4.28; N, 17.08; S, 7.97 %.

(E)-2-((1-Benzyl-6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9i**): 38 % yield; yellow solid; mp 236–238 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.79 (1H, s, H_9), 8.38 (1H, s, H_4), 8.33 (1H, br s, N*H*H), 8.14 (1H, br s, NH*H*), 7.51 (1H, d, J = 1.5 Hz, H_5), 7.35 (1H, dd, J = 1.6 and 8.6 Hz, H_7), 7.32–7.27 (3H, m, H_8, $H_{2'}$ and $H_{6'}$), 7.25–7.17 (3H, m, $H_{3'}$, $H_{4'}$ and $H_{5'}$), 5.54 (2H, s, H_{1a}), 2.34 (1H, s, CH_3) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.5, 137.1, 137.0, 136.6, 134.5, 132.5, 131.7, 129.1, 128.6, 127.0, 126.5, 124.5, 120.1, 115.2, 44.9, 20.0 ppm; v_{max}(neat, cm^{-1}): 3191 (NH), 3151 (NH), 1633 (C=O), 1522 (C=N), 1203 (C=S), 835 (C-S); HRMS (ESI) *m/z* (calcd for $C_{19}H_{18}N_4OS$: 350.1201, found 351.1273 $[M+H]^+$; Anal. calcd for $C_{19}H_{18}N_4OS{\cdot}0.25H_2O$: C, 64.29; H, 5.25; N, 15.78; S, 9.03 %. Found: C, 64.10; H, 5.24; N, 15.78; S, 9.22 %.

(E)-2-((6-Methyl-1-(4-nitrobenzyl)-2-oxo-1,2-dihydroquinolin-3-yl) methylene)hydrazinecarbothioamide (**9j**): 44 % yield; yellow solid; mp 241–243 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.83 (1H, s, H_9), 8.37 (1H, s, H_4), 8.35 (1H, br s, N*H*H), 8.17–8.16 (2H, m, $H_{3'}$ and $H_{5'}$), 8.12 (1H, br s, N*H*H), 7.52 (1H, d, J = 1.3 Hz, H_5), 7.47–7.43 (2H, m, $H_{2'}$ and $H_{6'}$), 7.37 (1H, dd, J = 1.7 and 8.7 Hz, H_7), 7.27 (1H, d, J = 8.7 Hz, H_8) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.5, 146.6, 144.7, 136.9, 136.8, 134.8, 132.7, 132.0, 129.3, 127.7, 124.5, 123.8, 120.2, 114.9, 44.7, 20.0 ppm; v_{max}(neat, cm^{-1}): 3215 (NH), 3150 (NH), 1638 (C=O), 1520 (C=N), 1205 (C=S), 850 (C-S); HRMS (ESI) *m/z* calcd for $C_{19}H_{17}N_5O_3S$: 395.1052, found 396.1135 $[M+H]^+$; Anal. calcd for $C_{19}H_{17}N_5O_3S{\cdot}0.25H_2O$: C, 57.06; H, 4.41; N, 17.51; S, 8.02 %. Found: C, 56.78; H, 3.98; N, 17.11; S, 7.98 %.

(E)-2-((2-Oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9k**): 59 % yield; yellow solid; mp 258 °C (Decomposed); δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.83 (1H, s, H_9), 8.36 (1H, br s, N*H*H), 8.32 (1H, s, H_4), 8.14 (1H, br s, NH*H*), 7.72 (1H, m, H_7 and H_8), 7.61 (1H, d, J = 8.5 Hz, H_5), 7.36 (1H, t, J = 7.95 Hz, H_6), 5.13 (2H, d, J = 1.9 Hz, H_{1a}), 3.29 (1H, t, J = 2.2 Hz, H_{2e}); δ_C(101 MHz, DMSO-d_6): 178.1, 159.6, 138.2, 136.7, 136.6, 131.4, 129.6, 124.4, 123.0, 120.1, 115.1, 78.6, 74.6, 31.4 ppm; v_{max}(neat, cm^{-1}): 3272 (NH), 3156 (NH), 1645 (C=O), 1532 (C=S), 846 (C-S); HRMS *m/z* calcd for $C_{14}H_{12}N_4OS$ 284.0732, found: 285.0819 $[M+H]^+$; Anal. calcd for $C_{14}H_{12}N_4OS{\cdot}0.125H_2O$: C, 58.67; H, 4.31; N, 19.55; S, 11.19 %. Found: C, 58.69; H, 3.81; N, 19.38; S, 11.15 %.

(E)-2-((6-methoxy-2-oxo-1-(prop-2-ynyl)-1,2-dihydroquinolin-3-yl) methylene)hydrazinecarbothioamide (**9l**): 56 % yield; yellow solid; mp 232 °C (Decomposed); δ_H(400 MHz; DMSO-d_6): 11.6 (1H, s, N(11)H), 8.70 (1H, s, H_9), 8.30 (1H, br s, N*H*H), 8.32 (1H, s, H_4), 8.09 (1H, br s, NH*H*), 7.46 (1H, d, J = 9.3 Hz, H_8), 7.25 (1H, dd, J = 2.9 and 9.2 Hz, H_7), 7.15 (1H, d, J = 2.9 Hz, H_5), 5.03 (2H, d, J = 2.1 Hz, H_{1a}), 3.75 (3H, s, CH_3), 3.18 (1H, t, J = 2.2 Hz, H_{2e}) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 159.1, 154.6, 136.7, 134.3, 132.8, 124.8, 120.8, 120.1, 116.5, 110.9, 78.8, 74.5, 55.4, 31.4 ppm; v_{max}(neat, cm^{-1}): 3258 (NH), 3172 (NH), 1635 (C=O), 1203 (C=S), 1521 (C=N), 849 (C-S); HRMS (ESI) *m/z* calcd for $C_{15}H_{14}N_4O_2S$: 298.0888, found 299.0805 $[M+H]^+$; Anal. calcd for $C_{15}H_{14}N_4O_2S$: C, 56.94; H, 5.10; N, 17.71; S, 10.14 %. Found: C, 56.52; H, 5.19; N, 17.58; S, 10.33 %.

(E)-2-((6-Methyl-2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinolin-3-yl) methylene)hydrazinecarbothioamide (**9m**): 60 % yield; yellow solid; mp 244 °C (Decomposed); δ_H (400 MHz; DMSO-d_6): 11.6 (1H, s, N(11)H), 8.75 (1H, s, H_9), 8.35 (1H, br s, NHH), 8.32 (1H, s, H_4), 8.07 (1H, br s, NH*H*), 7.55 (1H, d, J = 9.3 Hz, H_8), 734 (1H, dd, J = 2.9 and 9.2 Hz, H_7), 7.23 (1H, d, J = 2.9 Hz, H_5), 5.11 (1H, d, J = 2.1 Hz, H_{1a}), 3.25 (1H, t, J = 2.3 Hz, H_{2e}), 2.39 (3H, s, CH_3) ppm; δ_C (101 MHz, DMSO-d_6): 178.1, 159.4, 136.8, 136.2, 134.6, 132.6, 132.1, 129.1, 124.3, 120.0, 115.0, 78.8, 74.6, 31.3, 20.1 ppm; v_{max}(neat, cm^{-1}): 3279 (NH), 3183 (NH), 1627 (C=O), 1522 (C=N), 1206 (C=S), 836 (C-S); HRMS (ESI) *m/z* calcd for $C_{15}H_{14}N_4OS$: 314.0837, found 315.0927 $[M+H]^+$; Anal. calcd for $C_{15}H_{14}N_4OS{\cdot}0.75CH_3OH{\cdot}1H_2O$: C, 55.57, H; 5.63, N; 16.46, S; 9.42 %. Found; C; 55.32, H; 5.58, N; 16.8, S; 9.98 %.

(E)-2-((1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-2-oxo-1,2-dihydro quinolin-3-yl)methylene)hydrazinecarbothioamide (**9n**): 68 yield; yellow solid; mp 235–237 °C; δ_H(400 MHz; DMSO-d_6): 12.6 (1H, s, NH), 8.80 (1H, s, H_9), 8.44 (1H, d, J = 5.3 Hz, $H_{2'}$), 8.35 (1H, s, H_4), 8.33 (1H, br s, N*H*H) 8.11 (1H, brs, NHH), 8.06 (1H, d, J = 9.1 Hz, $H_{5'}$), 7.97 (1H, d, J = 1.9 Hz, $H_{8'}$), 7.71 (1H, d, J = 7.6 Hz, H_5), 7.60 (1H, t, J = 8.6 Hz, H_7), 7.57 –7.47 (2H, m, H_5 and NH), 7.45 (1H, dd, J =1.9 and 9.1 Hz, $H_{6'}$), 7.28 (1H, t, J = 7.4 Hz, H_6), 6.72 (1H, d, J = 5.4 Hz, $H_{3'}$), 4.56 (2H, t, J = 6.6 Hz, CH_2), 4.09 (2H, q, J = 6.0, 7.5 and 8.8 Hz, CH_2) ppm; δ_C(101 MHz, DMSO-d_6): 178.7, 161.2, 152.5, 150.5, 149.5, 139.7, 137.4, 133.4, 131.9, 130.2, 128.0, 124.9, 124.8, 124.7, 124.2, 123.2, 120.7, 114.9, 99.1, 49.1, 41.1 ppm; v_{max}(neat, cm^{-1}): 3234 (NH), 3150 (NH), 1633 (C=O), 1515 (C=N), 1204 (C=S), 844 (C-S), 716 (C-Cl); HRMS (ESI) *m/z* calcd for $C_{22}H_{19}ClN_6OS$: 450.1030, found 451.1099 $[M+H]^+$; Anal. calcd. for $C_{22}H_{19}ClN_6OS$: C, 58.60; H, 4.25; N, 18.64; S, 7.11 %, Found: C, 59.21; H, 4.22; N, 18.55; S, 6.99 %.

(E)-2-((1-(3-((7-Chloroquinolin-4-yl)amino)propyl)-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9o**): 48 % yield; amorphous yellow solid; mp 240–242 °C; δ_H(400 MHz; DMSO-d_6): 11.6 (1H, s, N(11)H), 8.78 (1H, s, H_9), 8.38 (1H, d, J = 5.3 Hz, $H_{2'}$), 8.34 (1H, s, H_4), 8.32 (1H, br s, N*H*H), 8.25 (1H, d, J = 9.0 Hz, $H_{5'}$), 8.10 (1H, br s, NHH), 7.79 (1H, d, J = 1.8 Hz, $H_{8'}$), 7.72 (1H, d, J = 1.0 and 7.7 Hz, H_8), 7.63 (1H, d, J = 0.7 and 8.6 Hz, H_5), 7.55 (1H, ddd, J = 1.0, 7.2 and 9.0 Hz, H_6), 7.46 (1H, dd, J = 1.8 and 9.0 Hz, $H_{6'}$), 7.39 1H, s, NH), 7.30 (1H, ddd, J = 0.7, 7.2 and 8.6Hz, H_7), 6.50 (1H, d, J = 5.4 Hz, $H_{3'}$), 4.47–4.39 (2H, m, $H_{1'}$), 3.45–3.41 (2H, m, $H_{3'}$), 2.10–2.01 (2H, m, $H_{2'}$) ppm; δ_C (101 MHz, DMSO-d_6): 178.6, 165.3, 160.6, 152.4, 150.5, 139.3, 137.6, 133.9, 130.2, 128.0, 124.9, 124.9, 124.7, 124.6, 124.4, 124.4, 123.1, 120.7, 118.0, 115.1, 99.2, 40.8, 39.3, 26.7 ppm; v_{max}(neat, cm^{-1}): 3325 (NH), 3234 (NH), 1641 (C=O), 1505 (C=N), 1194 (C=S), 854 (C-S), 712 (C-Cl); HRMS (ESI) *m/z* calcd for $C_{23}H_{21}ClN_6OS$: 464.1186, found 465.1268 [M+H]$^+$; Anal. calcd for $C_{23}H_{21}ClN_6OS$: C, 59.41; H, 4.55; N, 18.07; S, 6.90 %. Found: C, 59.82; H, 4.44; N, 17.93; S, 6.63 %.

(E)-2-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9p**): 45 % yield; yellow solid; mp 240–242 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.81 (1H, s, H_9), 8.35 (1H, s, H_4), 8.32 (1H, br s, N*H*H), 8.11 (1H, br s, NH*H*), 8.08 (1H, s, $H_{5'}$), 7.73–7.70 (2H, m, $H_{2'}$ and $H_{6'}$), 7.38 (1H, ddd, J = 1.2 and 7.3, 9.0 Hz, H_6), 7.37–7.24 (6H, m, H_5, H_7, H_8, $H_{5''}$, $H_{4'}$ and $H_{3'}$), 5.55 (2H, s, H_{4a}), 5.51 (2H, s, H_{1a}) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.2, 142.8, 138.9, 136.9, 135.4, 134.7, 131.3, 129.6, 128.7, 128.1, 127.9, 124.5, 123.7, 122.7, 120.1, 115.1, 52.7, 37.7 ppm; v_{max}(neat, cm^{-1}): 3215 (NH), 3156 (NH), 1634 (C=O), 1524 (C=N), 1567 (N=N), 1203 (C=S), 843 (C-S); HRMS (ESI) *m/z* calcd for $C_{21}H_{19}N_7OS$: 417.1372, found 418.1455 [M+H]$^+$; Anal. calcd for $C_{21}H_{19}N_7OS{\cdot}0.125H_2O$: C, 60.09; H, 4.62; N, 23.36; S, 7.64 %. Found: C, 60.04; H, 4.52; N, 22.99; S, 7.34 %.

(E)-2-((1-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9q**): 55 % yield; yellow solid; mp 258–260 °C; δ_H(400 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 8.80 (1H, s, H_9), 8.35 (1H, s, H_4), 8.33 (1H, br s, N*H*H), 8.22–8.18 (2H, m, $H_{3''}$ and $H_{5''}$), 8.17 (1H, s, $H_{5'}$), 8.11 (1H, s, NH*H*), 7.74–7.70 (2H, m, H_5 and H_8), 7.62 (1H, ddd, J = 1.3, 7.2 and 8.9 Hz, H_6), 7.52–7.47 (2H, m, $H_{2''}$ and $H_{6''}$), 7.31 (1H, ddd, J = 0.5, 7.2 and 8.4 Hz, H_7), 5.71 (2H, s, H_{4a}), 5.58 (2H, s, H_{1a}) ppm; δ_C(101 MHz, DMSO-d_6): 178.1, 160.3, 147.3, 143.4, 143.1, 138.9, 136.9, 134.8, 131.5, 129.2, 124.6, 124.3, 123.9, 122.9, 120.2, 51.9 ppm; v_{max}(neat, cm^{-1}): 3361 (NH), 3255 (NH), 1635 (C=O), 1514 (C=N), 1564 (N=N), 1202 (C=S), 847 (C-S); HRMS (ESI) *m/z* calcd for $C_{21}H_{18}N_8O_3S$: 462.1223, found 463.1306 [M+H]$^+$; Anal. calcd for $C_{21}H_{18}N_8O_3S{\cdot}0.5H_2O$: C, 53.50; H, 4.06; N, 23.77; S, 6.80 %. Found: C, 53.33; H, 3.77; N, 23.95; S, 6.58 %.

(E)-2-((1-((1-(7-Chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-2-oxo-1,2-dihydroquinolin-3-yl)methylene)hydrazinecarbothioamide (**9r**): 46 % yield; amorphous yellow solid; mp 251–253 °C; δ_H(300 MHz; DMSO-d_6): 11.7 (1H, s, N(11)H), 9.10 (1H, d, J = 4.6 Hz, $H_{2''}$), 8.84 (1H, s, H_9), 8.77 (1H, s, $H_{5'}$), 8.38 (1H, s, H_4), 8.33 (1H, br s, N*H*H), 8.26 (1H, d, J = 1.8 Hz, $H_{8'}$), 8.13 (1H, br s, NH*H*), 7.97 (1H, d, J = 8.9 Hz, $H_{5'}$), 7.85–7.72 (4H, m, H_5, H_8, $H_{3'}$ and $H_{6'}$), 7.67 (1H, ddd, J = 1.3, 7.0 and 8.9 Hz, H_6), 7.34 (1H, ddd, J = 0.6, 7.0, 8.6 Hz, H_7), 5.76 (2H, s, H_{4a}) ppm; δ_C(75 MHz, DMSO-d_6): 178.1, 160.2, 152.9, 149.3, 143.5, 140.2, 139.0, 136.9, 136.3, 134.8, 131.4, 129.7, 128.9, 128.1, 125.9, 125.4, 124.6, 122.8, 120.2, 117.0, 115.2, 37.5 ppm; v_{max}(neat, cm^{-1}): 3358 (NH), 3233 (NH), 1638 (C=O), 1517 (C=N), 1568 (N=N), 1516 (C=N), 1205 (C=S), 851 (C-S); HRMS (ESI) *m/z* calcd for $C_{23}H_{17}ClN_8OS$: 488.0935, found 489.1011 [M+H]$^+$. Anal. calcd for $C_{23}H_{17}ClN_8OS{\cdot}0.25H_2O$: C, 55.98; H, 3.57; N, 22.71; S, 6.50 %. Found: C, 55.81; H, 3.46; N, 23.01; S, 6.62 %.

4.3. Biological Testing and Growth Inhibition Assays

4.3.1. *In Vitro Antitrypanosomal and Cytotoxicity Assays*

The HeLa cells (Cellonex) were cultured using method described by Oderinlo *et al*. and Adeyemi *et al*.[30,31] *Trypanosoma brucei brucei* 427 trypomastigotes were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Lonza) supplemented with 10 % fetal calf serum, HMI-9 supplement,[32] hypoxanthine and penicillin/streptomycin at 37 °C in a 5 % CO_2 incubator. Serial dilutions of test compounds were incubated with the parasites in 96-well plates for 24 h and residual parasite viability in the wells determined by adding 20 μL of 0.54 mM resazurin in phosphate buffered saline (PBS) and incubating for an additional 24 h. Reduction of resazurin to resorufin by viable parasites was assessed by fluorescence readings (excitation 560 nm, emission 590 nm) in a Spectramax M3 plate reader. Fluorescence readings were converted to % parasite viability relative to the average readings obtained from untreated control wells. IC_{50} values were determined by plotting % viability *vs.* log[compound] and performing non-linear regression using GraphPad Prism (v. 5.02) software.[30,31]

4.3.2. *In Vitro Antiplasmodial Assay*

Activity was determined against the 3D7 chloroquine-sensitive strain of *P. falciparum*. Parasites were maintained in continuous culture using the method of Trager and Jensen[33] with modifications. Growth medium consisted of RPMI 1640 containing 25 mM HEPES, and further supplemented with 0.5 % (w/v) Albumax II, 22 mM glucose, 0.65 mM hypoxanthine, 0.05 mg mL^{-1} gentamicin and 2–4 % (v/v) human erythrocytes. Parasites were cultured at 37 °C under an atmosphere of 5 % CO_2, 5 % O_2, 90 % N_2.[30,31] Compounds were prepared as 20 mM stock solutions in dimethyl sulfoxide, sonicated for 10 minutes to enhance solubility and stored at –20 °C until use. To assess antimalarial activity, compounds were diluted to a final concentration of 20 μM in culture medium, added to parasite cultures (2 % parasitaemia, 1 % haematocrit) in 96 well plates and incubated for 48 h at 37 °C under an atmosphere of 5 % CO_2, 5 % O_2, 90 % N_2. Parasite viability was assessed using the parasite lactate dehydrogenase assay described by Makler *et al*.[34] Wells containing uninfected erythrocytes were used as negative controls (0 % parasite viability) and untreated parasite-infected wells as positive controls (100 % parasite viability). To determine IC_{50}-values, parasite cultures were incubated with 3-fold serial dilutions of test compounds and non-linear regression analysis carried out on dose-response plots of % parasite viability *vs.* log[compound] using GraphPad Prism (v. 5.02) software.

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