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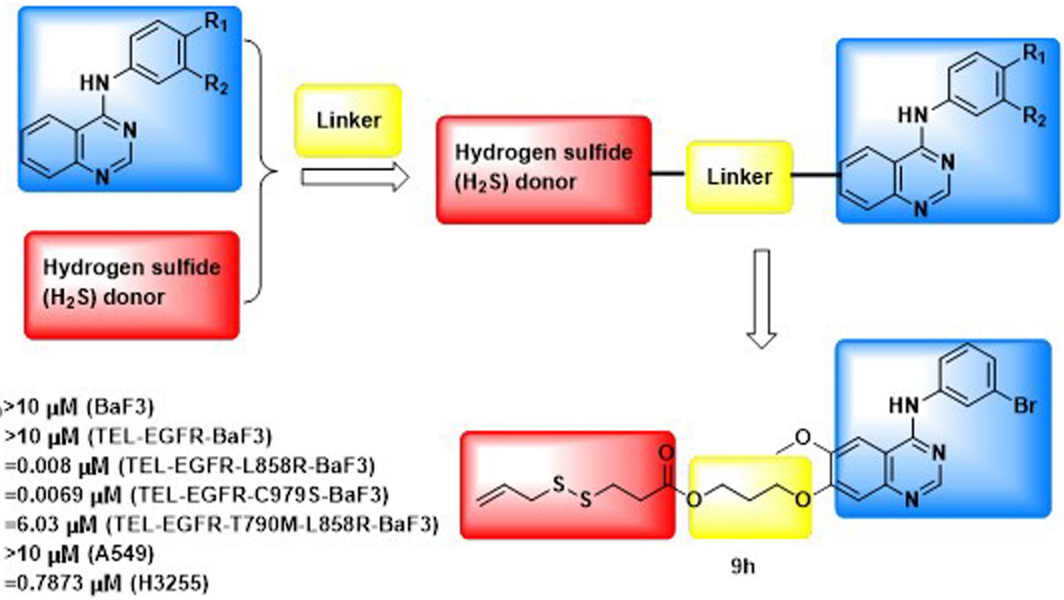

GI_{50}>10 μM (BaF3)
>10 μM (TEL-EGFR-BaF3)
=0.008 μM (TEL-EGFR-L858R-BaF3)
=0.0069 μM (TEL-EGFR-C979S-BaF3)
=6.03 μM (TEL-EGFR-T790M-L858R-BaF3)
>10 μM (A549)
=0.7873 μM (H3255)

9h

Design, Synthesis and Biological Evaluation of 4-Aniline Quinazoline Derivatives Conjugated with Hydrogen Sulfide (H_2S) Donors as Potent EGFR Inhibitors against L858R Resistance Mutation

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ABSTRACT

In this study, a series of 4-aniline quinazoline derivatives bearing hydrogen sulfide (H_2S) donors were designed, synthesized and evaluated for biological activities. The synthesized compounds were screened for the enzymatic activities against EGFR and EGFR mutants by kinase target-based cell screening method. The results demonstrate that most compounds exhibit selectively inhibitory activities against TEL-EGFR-L858R-BaF3, especially compound **9h** with GI_{50}=0.008 μM (TEL-EGFR-L858R-BaF3), 0.0069 μM (TEL-EGFR-C797S-BaF3), >10 μM (BaF3), >10 μM (TEL-EGFR-BaF3) and 6.03 μM (TEL-EGFR-T790M-L858R-BaF3). The results from anti-proliferative assays in two NSCLC cell lines indicate that synthetic derivatives (**9g**, **9h**, **15e** and **15f**) with H_2S donor ACS81 display greater anti-proliferative potency against NSCLC cell line H3255 bearing EGFR mutant (L858R) with GI_{50} values ranging from 0.3486 to 1.348 μM. In addition, compound **9h** exhibits weak anti-proliferative effects on other tumor cells (HepG2, MCF-7, HT-29 and A431) and has lower toxic effect on HUVEC cells than AZD9291 (positive control). Meanwhile, compound **9h** inhibits the

phosphorylation of EGFR in H3255 cells in a dose-dependent manner. Cell cycle analysis reveals that compound **9h** suppresses the proliferation of cells by inducing cell cycle arrest in G0-G1 phase. The result of H_2S release evaluation suggests that the H_2S release of compound **9h** is significantly more and faster than other compounds.

Keywords: EGFR, Conjugate, Hydrogen Sulfide, H3255 (L858R)

1. Introduction

Quinazoline alkaloids are nitrogen-containing heterocycles with a broad range of biological properties, such as anticancer[1, 2], anticonvulsant[3], antifungal[4], anti-inflammatory[5], antimalaria[6], antibacterial[7], antihypertensive[8], anti-HIV[9] activities. Many quinazoline derivatives have been developed to bind to various targets, including epidermal growth factor receptor (EGFR)[10-13], vascular endothelial growth factor receptor (VEGFR)[14], janus kinase 2 (JAK2)[15], histone deacetylase (HDAC)[16], Bromodomain-containing protein 4 (BRD4)[17], cyclin-dependent kinases (CDK)[18], poly ADP-ribose polymerase (PARP)[19], MEK5/ERK5 pathway[20], and so on. In our previous work, we have reported several 4-anilinoquinazoline derivatives, which show significant activity against cancer cells [21-23]. On the basis of the literature and our preliminary studies, we used 4-anilinoquinazoline as the scaffold to design new antitumor agents in this study.

Hydrogen sulfide (H_2S) was known as toxic pollutant for many years. However, it is now widely recognized as an important biological and pharmacological molecule. In the past decade, studies reveal that H_2S plays critical role in physiology and pathology[24]. In cancer, H_2S has been showed to exhibit remarkable *in vitro* antiproliferative activity against tumor cell lines, such as A549/DDP[25], HepG2[26], and MCF-7[27]. Although H_2S exerts crucial effects on antitumor, it cannot be used as an anticancer agent due to the uncontrollability and toxicity[28]. Meanwhile, several slow-releasing H_2S donors were reported to display antitumor effect [26, 29], and many H_2S donors were discovered (Fig. 1). In addition, previous relevant studies also demonstrate that the compounds bearing H_2S donors have potent inhibitory activities against tumor cells [28, 30-33].

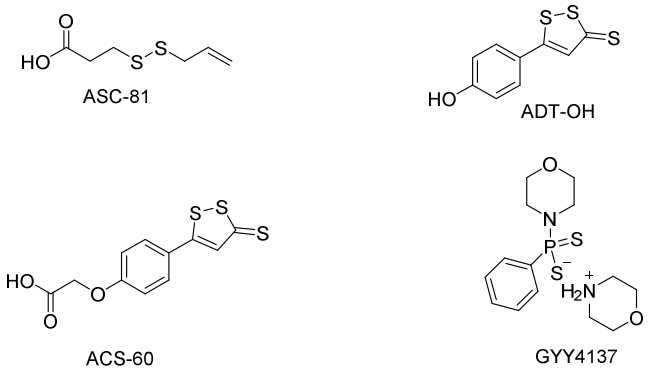


Fig. 1. Hydrogen sulfide (H_2S) donors

In the light of above findings and our previous studies, we designed and synthesized of a series novel 4-anilinoquinazoline derivatives linked with various H_2S donors on position 6 or 7 of quinazoline ring through different chains (Fig. 2).

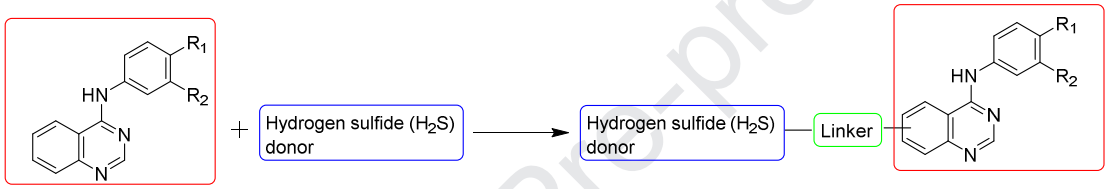
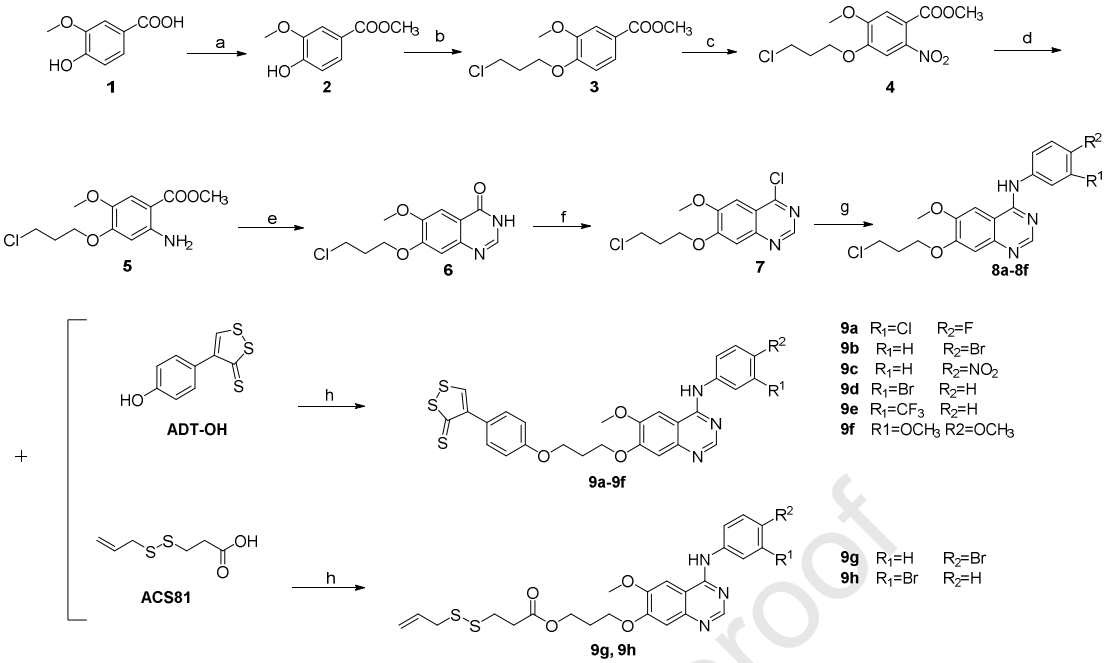


Fig. 2. Design of novel 4-aniline quinazoline derivatives bearing hydrogen sulfide (H_2S) donors

2. Results and discussion

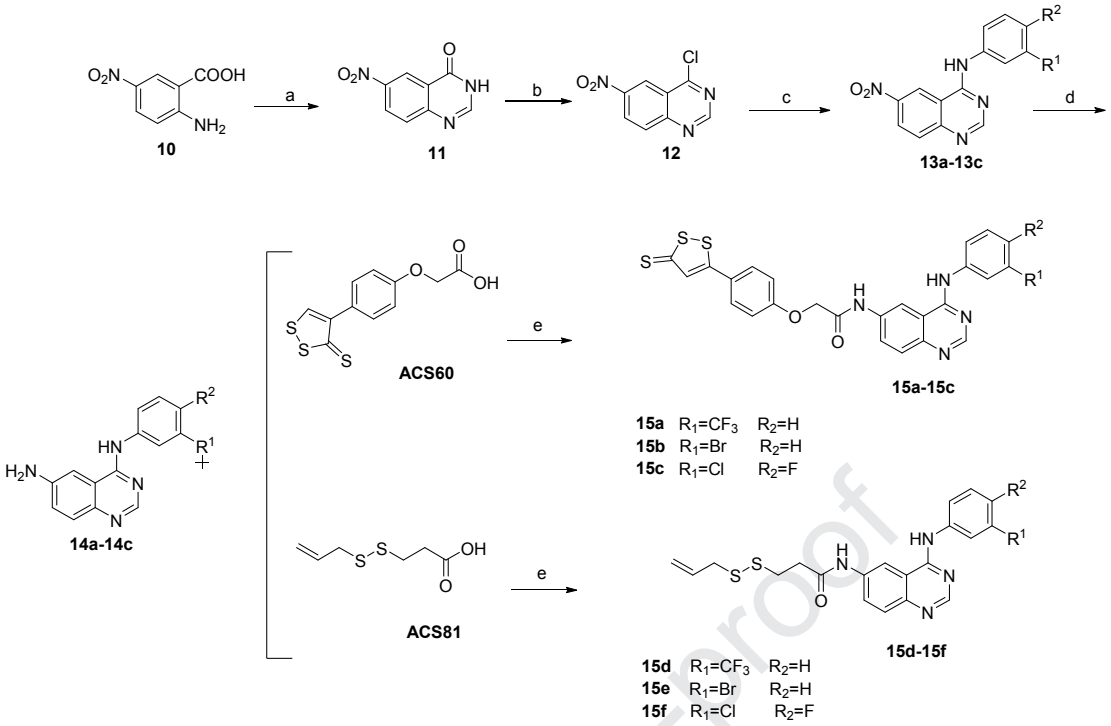
2.1. Chemistry

The overall synthetic route of compounds (**9a-9h)** was outlined in Scheme 1 starting from vanillic acid. After the esterification, alkylation, nitration, reduction, cyclisation and chlorination, compounds (**8a-8f**) were obtained. The target compounds (**9a-9h**) were prepared by reacting compound (**8**) with two H_2S donors.



Scheme 1 The synthesis of compounds **9a-9h**. Reagents and conditions: (a)CH_3OH, 98% sulfuric acid, reflux; (b)K_2CO_3, KI, 1-bromo-3-chloropropane, DMF, rt; (c) nitric acid, acetic acid, acetic anhydride, 0-5 °C; (d) Pd/C, methanol, rt; (e) formamidine acetate, alcohol, reflux; (f) $SOCl_2$, DMF, reflux; (g) substituted aniline, isopropanol, reflux; (h) K_2CO_3, KI, ADT-OH or ACS81, DMF rt.

As depicted in Scheme 2, 2-amino-5-nitrobenzoic acid (**10**) was converted by cyclisation with formamidine acetate to obtain compound (**11**), which was chlorinated by thionyl chloride to afford the chloride compound (**12**). Compound (**12**) was treated with substituted aniline in isopropanol to give compounds (**13a-13c**). Then compounds (**13a-13c**) were reduced to their amino derivatives (**14a-14c**) using iron powder. The target compounds (**15a-15f**) were synthesized by reacting compounds (**14a-14c**) with two H_2S donors using HATU.



Scheme 2 The synthesis of compounds **15a-15f**. Reagents and conditions: (a)formamidine acetate, 2-methoxyethanol, reflux; (b)$SOCl_2$, DMF, reflux; (c) substituted aniline, isopropanol, reflux; (d) iron powder, acetic acid/ethanol, refiux; (e) HATU, triethylamine, ACS60 or ACS81, rt.

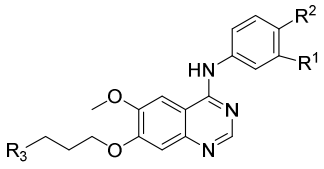
2.2. Biological activities

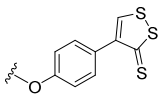
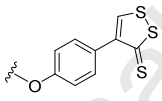
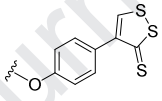
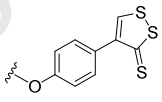
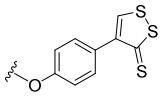
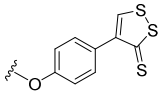
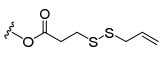
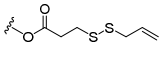
2.2.1. *In vitro* inhibition activities studies of kinases

Many novel 4-aniline quinazoline derivatives target EGFR, which is closely associated with cancer[34-36]. Therefore, the target compounds were assayed with the enzymatic activities against EGFR and EGFR mutants by kinase target-based cell screening method [37, 38]. As showed in Table 1, most of the compounds (**9a-9h**) displayed selective anti-proliferation efficacy against TEL-EGFR-L858R-BaF3 (GI_{50}=0.008-0.85μM), while having poor activities against BaF3, TEL-EGFR-BaF3 and TEL-EGFR-T790M-L858R-BaF3, especially compound **9h** with GI_{50}=0.008 μM (TEL-EGFR-L858R-BaF3), >10 μM (BaF3), >10 μM (TEL-EGFR-BaF3) and 6.03 μM (TEL-EGFR-T790M-L858R-BaF3). Compounds bearing H_2S donor ACS81 showed better inhibiting activities (GI_{50}=0.008-0.058μM) than others with ADT-OH (GI_{50}>0.10 μM). Meanwhile, compounds with *m*-Br in 4-aniline group exhibited greater potency against TEL-EGFR-L858R-BaF3, such as compound **9h**

(GI_{50}=0.008μM) and **9d** (GI_{50}=0.12 μM). In addition, compound **9h** was tested against TEL-EGFR-C797S-BaF3 with GI_{50}=0.0069μM, so there was no interaction between the allyl-disulfanyl-propionic group and the C797 residue, and the biochemical evaluation of the mechanism of the compound **9h** is being further investigated.

Table 1 The activities of the compounds (**9a-9h**) against EGFR and EGFR mutations (GI_{50} μM)

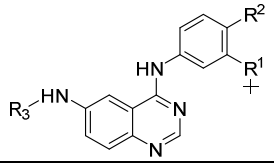


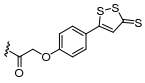
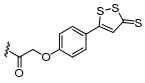
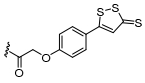
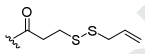
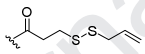
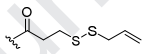
Compd	R_1	R_2	R_3	BaF3	TEL-EGFR-BaF3	TEL-EGFR-L858R-BaF3	TEL-EGFR-T790M-L858R-BaF3
9a	Cl	F		>10	>10	0.27	>10
9b	H	Br		9.23	9.56	0.25	>10
9c	H	NO_2		3.79	4.08	0.60	>10
9d	Br	H		9.23	3.06	0.12	>10
9e	CF_3	H		3.79	1.98	0.38	1.648
9f	$-OCH_3$	$-OCH_3$		2.65	3.39	0.83	6.35
9g	H	Br		3.91	2.34	0.058	>10
9h	Br	H		>10	>10	0.008	6.03
AZD 9291				2.6	0.28	<0.003	0.06

Several of compounds **15a-15f** were also tested the enzymatic activities against EGFR

and EGFR mutants. As showed in Table 2, the compounds with ACS81 also exhibited stronger inhibition against TEL-EGFR-L858R-BaF3, such as compound **15e** (GI_{50}=0.011μM) and compounds **15f** (GI_{50}=0.012μM).

Table 2 The activities of the compounds (**15a-15f**) against EGFR and EGFR mutations (GI_{50} μM)



Compd	R_1	R_2	R_3	BaF3	TEL-EGFR-BaF3	TEL-EGFR-L858R-BaF3	TEL-EGFR-T790M-L858R-BaF3
15a	CF_3	H		NT	NT	NT	NT
15b	Br	H		NT	NT	NT	NT
15c	Cl	F		4.27	2.49	0.29	3.16
15d	CF_3	H		NT	NT	NT	NT
15e	Br	H		＞10	＞10	0.011	＞10
15f	Cl	F		＞10	＞10	0.012	＞10
AZD 9291				2.6	0.28	<0.003	0.06

2.2.2. Anti-proliferation assay

To explore the potential antitumor effect, the *in vitro* anti-proliferative activities of the synthetic derivatives were evaluated against the NSCLC cell lines A549 (A549 harboring wide type EGFR) and H3255 (H3255 expressing EGFR-L858R). AZD9291(a third-generation EGFR inhibitor) that shows inhibiting EGFR phosphorylation in cells harboring sensitising EGFR mutants including H3255 (L858R), PC-9 (ex19del) and H1650 (ex19del) was choose as positive control. The results were summarized in Table 3. All of the target compounds showed poor anti-proliferative activities against A549 (GI_{50}>10 μM), while most compounds exhibited potent inhibitory activities against H3255 with the GI_{50} ranging from 0.3486 to 8.578 μM. Furthermore, compound **9g**, **9h**, **15e** and **15f** with H_2S donor ACS81

showed greater anti-proliferative potency against H3255 with GI_{50} values at 1.348, 0.7873, 0.5195 and 0.3486 μM, respectively. It seems that the result of anti-proliferative activities was consistent with their enzymatic inhibitory activities. Then the H3255 cells were treated with compound **9h** together with $ZnCl_2$ (10μM $ZnCl_2$ to quench the released H_2S), but the GI_{50} (GI_{50}=0.3028μM) of which decreased rather than increased compared with that of compound **9h** only. We guess that it may owe to the inhibitory activity of $ZnCl_2$ against H3255 (Cell proliferation rate was only 54.02% at 10 μM of $ZnCl_2$).

Table 3 *In vitro* anti-proliferative activities in two NSCLC cell lines of target compounds.

compd	A549 (GI_{50} μM)	H3255 (GI_{50} μM)
9a	>10	>10
9b	>10	1.408
9c	>10	1.874
9d	>10	2.404
9e	>10	7.472
9f	>10	8.578
9g	>10	1.348
9h	>10	0.7873
15a	>10	NT
15b	>10	NT
15c	>10	4.355
15d	>10	NT
15e	>10	0.5195
15f	>10	0.3486
$ZnCl_2$	NT	54.02%[a]
9h+$ZnCl_2$(10μM)	NT	0.3028
AZD9291	NT	0.09

a Cell proliferation rate (10 μM)

In addition, *in vitro* anti-proliferative activities against other four tumor cells (harboring wide type EGFR) of compound **9h** were tested. As described in Table 4, **9h** exhibits weak anti-proliferative effects on HepG2, MCF-7, HT-29 and A431 cell lines with IC_{50} value of >10, 7.309, >10 and 6.703 μM, respectively. To investigate the tumor selectivity, the cytotoxicity of **9h** on normal cells (HUVEC cells) was assayed. The result showed that **9h** has no toxic effect on HUVEC cells (IC_{50}>10μM).

Therefore, compound **9h** selectively inhibits NSCLC cell line H3255 (expressing EGFR-L858R) compared to other tumor cells and has lower toxic effect on HUVEC cells (IC_{50}>10μM) than AZD9291 (IC_{50}=7.278μM).

Table 4 *In vitro* anti-proliferative activities against other tumor cells and normal cells of compound **9h** (IC_{50} μM)

Compound	HepG2	MCF-7	HT-29	A431	HUVEC
9h	**>** 10	7.309	**>** 10	6.703	**>** 10
AZD9291	2.239	2.54	2.834	2.823	7.278

2.2.3. Compound **9h** inhibited EGFR phosphorylation in H3255 cells

In order to further verify the activities of compound **9h** in cellular level, we examined its effect on the phosphorylation of EGFR in the representative cancer cell line H3255, which was treated with various concentrations of **9h** for 4h. As shown in Fig. 3, compound **9h** inhibited the phosphorylation of EGFR in H3255 cells in a dose-dependent manner.

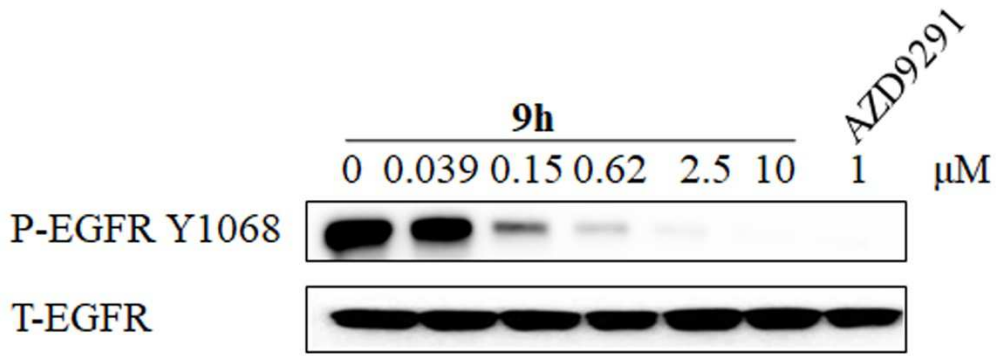


Fig. 3. Compound **9h** suppressed the phosphorylation of EGFR in H3255 cells

2.2.4. Cell cycle analysis

Previous studies reported that EGFR inhibitors can induce cell cycle arrest in tumor cells [39-42]. To determine the effect of compound **9h** on cell cycle, flow cytometry was performed on H3255 cells treated with various concentrations of **9h** for 72 h. As described in Fig. 4, the percentage of H3255 cells in G0-G1 phase increased from 60.32% to 88.61% after treatment of compound **9h** with 1, 5 and 10 μM, indicating that compound **9h** inhibits the proliferation of cells by inducing cell cycle arrest in

G0-G1 phase.

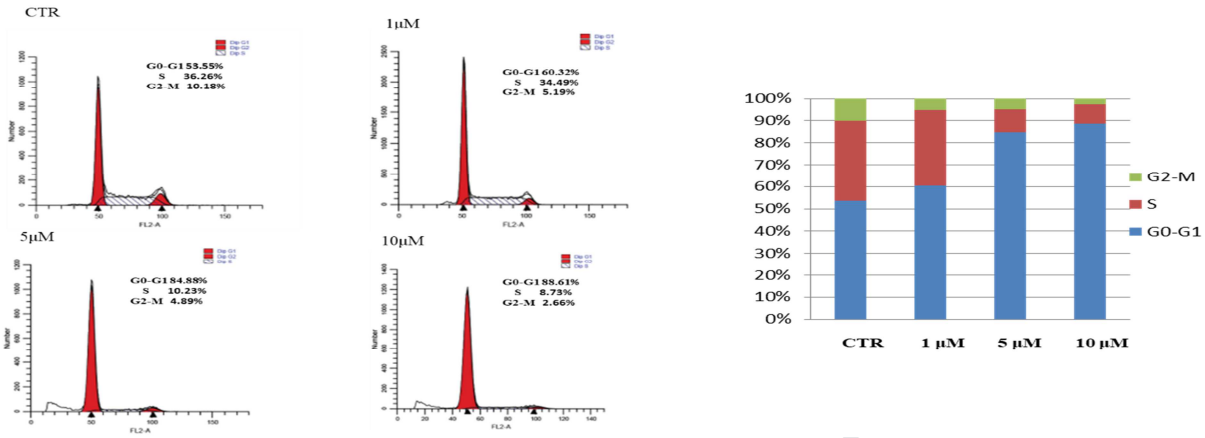


Fig. 4 **9h** induced G0-G1 phase cell cycle arrest on H 3255.

2.2.5 *In vitro* hydrogen sulfide release evaluation

Using the hydrogen sulfide probe (**NIR-HS**) developed by our group[43], compound **9f**, **9h** and **15c** bearing various H_2S donors were tested their ability to release hydrogen sulfide by the published method [44]. After incubating the compounds **9f**, **9h** and **15c** (10, 50, 100, 200 μM) with the fluorescent probe **NIR-HS** (10 μM), significant fluorescence signals were produced, and the fluorescence intensity increased significantly compared with that without the compounds (*P < 0.05,**P < 0.01) (Fig. 5). This indicates that all compounds can release H_2S in PBS buffer.

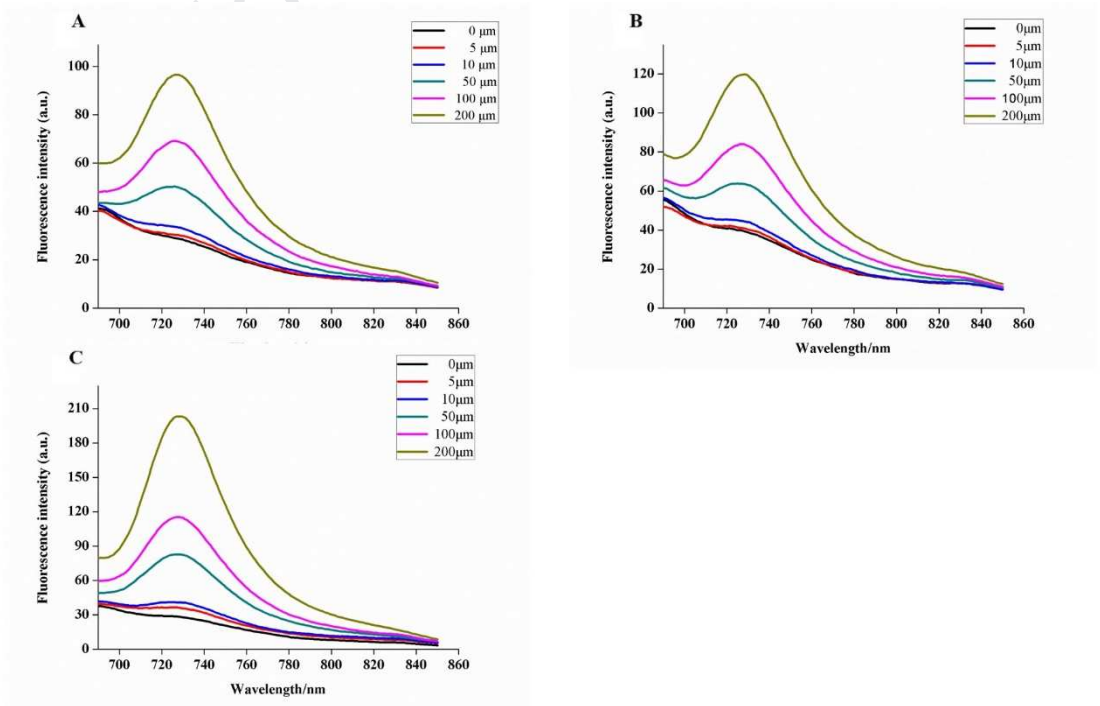


Fig. 5 Fluorescence emission spectra of NIR-HS (10μM) with varied concentrations of **9f** (A), **9h** (B) and **15c** (C) in PBS buffer (pH=7.4, 60% acetone) at 37□for 3 h.

Then quantitative determinations of H_2S release from target compounds were performed. As shown in Fig 6A and 6B, the standard curve between the concentration of Na_2S and fluorescence intensity of **NIR-HS** probe was established, which has excellent linear relationship. Release of H_2S by compound **9f**, **9h** and **15c** were quantitatively determined based on the standard curve. The results indicate that all the compounds could release H_2S, especially compound **9h** with the ability of H_2S releasing significantly more than that of compound **9f** and **15c**. Within 1h, the release rate of H_2S of compound **9h** was faster than other two compounds and tended to level off after 2h (Fig 6C). This result is consistent with the in vitro inhibition activities of kinases and cells.

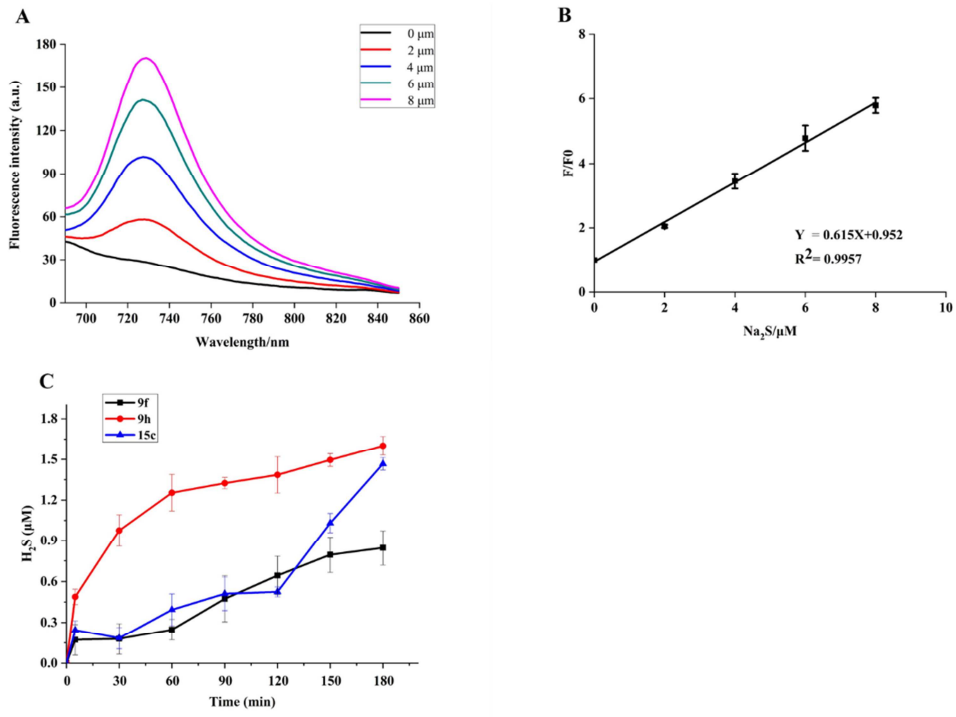


Fig. 6 (A) Fluorescence emission spectra of NIR-HS (10μM) with varied concentrations of Na_2S (0, 2, 4, 6, 8μM) in PBS buffer (pH=7.4, 60% acetone) at 37□

for 3h. (B) The fluorescence intensity (F/F_0) of the reaction system at 723 nm against the corresponding reagent blank (without Na_2S), which is the calibration curve for this study. (C) Time-dependent hydrogen sulfide-releasing derivatives of **9f**, **9h** and **15c**.

3. Conclusions

In summary, we designed and synthesized a series of 4-aniline quinazoline derivatives conjugated with hydrogen sulfide (H_2S) donors and the target compounds were assayed with the enzymatic activities against EGFR and EGFR mutations. Most compounds displayed inhibitory activities against TEL-EGFR-L858R-BaF3 and achieved selectivity over TEL-EGFR-BaF3 and TEL-EGFR-T790M-L858R-BaF3, especially compound **9h** with GI_{50}=0.008 μM (TEL-EGFR-L858R-BaF3), >10 μM (TEL-EGFR-BaF3) and 6.03 μM (TEL-EGFR-T790M-L858R-BaF3). Additionally, anti-proliferation assays showed that compounds (**9g**, **9h**, **15e** and **15f**) with H_2S donor ACS81 exhibited greater anti-proliferative potency against EGFR mutant NSCLC cell line H3255 (L858R) with GI_{50} values at 1.348, 0.7873, 0.5195 and 0.3486 μM respectively, and it is consistent with their enzymatic inhibitory activities. In addition, compound **9h** selectively inhibits NSCLC cell line H3255 (expressing EGFR-L858R) compared to other tumor cells (HepG2, MCF-7, HT-29 and A431 cell lines) and has lower toxic effect on normal cells (HUVEC) than AZD9291. Furthermore, compound **9h** inhibited the phosphorylation of EGFR in H3255 cells in a dose-dependent manner. Cell cycle analysis uncovered compound **9h** inhibited the proliferation of cells by inducing cell cycle arrest in G0-G1 phase. The result of hydrogen sulfide release evaluation exhibited that all the compounds could release H_2S, especially compound **9h** with the ability of H_2S releasing significantly more and faster than other compounds. Hence, compound **9h** merits further exploration as a potential candidate for the EGFR-L858R positive NSCLC.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all reagents were purchased from commercial sources and

used without further purification. All compounds were routinely monitored by thin-layer chromatography with silica gel GF-254 glass plates and viewed under UV light at 254 nm. The ^{1}H-NMR and ^{13}C-NMR spectra were determined in DMSO-d_6 on a JNM-ECZ400s/L spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard. The mass spectra (MS) were obtained from Agilent 1100LC/MS Spectrometry Services. HR-MS was obtained using a Q-tof high resolution mass spectrometer.

4.1.1. Synthesis of compounds (**2-8**)

Compounds **2-8** were prepared according to a similar procedure in Refs[23].

4.1.2. General procedure for the preparation of the compounds **9**

To a solution of compounds **8** (1mmol) in DMF (10 mL) was added K_2CO_3 (2mmol) and catalytic amount of KI, stirred for 10mins, followed by corresponding H_2S donors (1mmol). The reaction mixture was stirred at 80 ^{o}C and monitored by thin layer chromatography. After completion of the reaction, the mixture was cooled to rt, poured into ice water (100 mL), stirred for 30mins. The solid formed was filtered off and purified by a silica gel column chromatography to afford compound **9**.

4.1.2.1.

*4-(4-(3-((4-((3-chloro-4-fluorophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propoxy) phenyl)-3H-1,2-dithiole-3-thione (**9a**)*

Orange solid, yield: 20%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.91 (s, 1H), 7.64-7.59 (m, 2H), 7.31 (d, *J* = 16.0 Hz, 5H), 7.22-7.18 (m, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 4.41-4.31 (m, 4H), 4.04 (s, 3H), 2.46 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.2, 174.2, 172.5, 162.4, 156.5, 154.1, 153.0, 152.5, 149.6, 134.6, 129.5, 124.2, 124.0, 122.9, 122.8, 119.4, 119.2, 117.1, 116.9, 116.0, 109.2, 108.1, 102.4, 65.6, 65.3, 56.8, 28.7; LC-MS *m/z*: 585.8 $[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{27}H_{20}ClFN_3O_3S_3$([M-H]$^-$):584.0339 (100%), found:584.0362; Calcd for $C_{27}H_{20}ClFN_3O_3S_3$([M-H]$^-$):586.0310 (32%), found:586.0332; Calcd for $C_{27}H_{20}ClFN_3O_3S_3$([M-H]$^-$):585.0373 (29.2%), found:585.0380.

4.1.2.2.

*4-(4-(3-((4-((4-bromophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propoxy)phenyl)-3H-1,2-dithiole-3-thione (**9b**)*

Orange solid, yield: 16%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.63 (dd, *J* = 8.0, 12.0 Hz, 3H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.39 (s, 1H), 7.29 (d, *J* = 12.0 Hz, 3H), 7.07-7.01 (m, 3H), 4.39-4.29 (m, 4H), 4.03 (s, 3H), 2.47-2.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.7, 173.0, 169.7, 168.0, 162.2, 154.1, 149.7, 134.7, 132.0, 128.6, 124.3, 123.4, 121.8, 117.0, 116.2, 115.5, 100.7, 65.3, 64.7, 56.4, 29.7; LC-MS *m/z*: 611.8 $[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{27}H_{21}BrN_3O_3S_3$ ($[M-H]^-$): 609.9928(100%), found:609.9918, Calcd for $C_{27}H_{21}BrN_3O_3S_3$ ([M-H]-): 611.9908(97.3%), found: 611.9930.

4.1.2.3.

*4-(4-(3-((6-methoxy-4-((4-nitrophenyl)amino)quinazolin-7-yl)oxy)propoxy)phenyl)-3H-1,2-dithiole-3-thione (**9c**)*

Orange solid, yield: 19%. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.05 (s, 1H), 8.65 (s, 1H), 8.30 (d, *J* = 8.0 Hz, 2H), 8.20 (d, *J* = 12.0 Hz, 2H), 7.92-7.87 (m, 2H), 7.81-7.76 (m, 1H), 7.31 (s, 1H), 7.13 (dd, *J* = 4.0 Hz, 2H), 4.37-4.29 (m, 4H), 4.01 (s, 3H), 2.33-2.30 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.3, 174.2, 162.4, 162.0, 156.2, 154.5, 152.6, 149.92, 142.1, 134.7, 129.5, 128.7, 125.1, 124.2, 121.2, 116.6, 116.0, 109.9, 108.0, 102.5, 65.7, 65.3, 56.9, 28.7; LC-MS *m/z*: 578.9 $[M+H]^+$.

4.1.2.4.

*4-(4-(3-((4-((3-bromophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propoxy)phenyl)-3H-1,2-dithiole-3-thione (**9d**)*

Orange solid, yield: 20%.^{1}H NMR (400 MHz, d_6-DMSO) δ 9.53 (s, 1H), 8.53 (s, 1H), 8.16 (s, 1H), 7.90-7.75 (m, 5H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 16.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 4.33-4.28 (m, 4H), 3.98 (s, 3H), 2.30 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.3, 174.2, 162.4, 156.5, 154.0, 153.1, 149.6, 147.3, 141.7,

134.6, 130.8, 129.5, 128.7, 126.1, 124.5, 124.2, 121.7, 121.1, 116.0, 115.8, 109.4, 108.3, 102.4, 65.6, 65.3, 56.8, 28.7; LC-MS *m/z*: 611.7 $[M+H]^+$ (100%); MS (*m/z*): HRMS(ESI) Calcd for $C_{27}H_{21}BrN_3O_3S_3$([M-H]$^-$): 611.9908(97.3%), found: 611.9960.

4.1.2.5.

*4-(4-(3-((6-methoxy-4-((3-(trifluoromethyl)phenyl)amino)quinazolin-7-yl)oxy)propoxy)phenyl)-3H-1,2-dithiole-3-thione (**9e**)*

Orange solid, yield: 20%. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.67 (s, 1H), 8.54 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 3H), 7.75 (s, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.26 (s, 1H), 7.13 (d, *J* = 12.0 Hz, 2H), 4.35-4.28 (m, 4H), 4.00 (s, 3H), 2.32-2.29 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.2, 174.2, 170.8, 162.4, 156.5, 154.1, 153.0, 149.6, 147.4, 140.9, 134.6, 130.0, 129.5, 126.1, 125.9, 124.2, 123.4, 119.8, 118.3, 116.0, 109.5, 108.4, 102.4, 65.6, 65.3, 56.8, 28.7; LC-MS *m/z*: 601.8 $[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{28}H_{23}F_3N_3O_3S_3$([M+H]$^+$): 602.0854, found:602.0830.

4.1.2.6.

*4-(4-(3-((4-((3,4-dimethoxyphenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propoxy)phe nyl)-3H-1,2-dithiole-3-thione (**9f**)*

Orange solid, yield: 17%.^{1}H NMR (400 MHz, d_6-DMSO) δ 9.39 (s, 1H), 8.42 (s, 1H), 7.90-7.83 (m, 3H), 7.75 (s, 1H), 7.38 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.21 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.99 (d, *J* = 12.0 Hz, 1H), 4.32-4.29 (m, 4H), 3.97 (s, 3H), 3.79 (d, *J* = 8.0 Hz, 6H), 2.32-2.29 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.3, 174.2, 162.4, 157.1, 153.7, 149.3, 148.9, 145.88, 134.6, 133.0, 129.5, 128.7, 116.5, 116.0, 115.8, 115.6, 112.3, 109.2, 108.6, 108.1, 102.7, 65.5, 65.3, 56.8, 56.2, 56.1, 28.8; LC-MS *m/z*: 593.8$[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{29}H_{28}N_3O_5S_3$ ($[M+H]^+$): 594.1191, found:594.1163; Calcd for $C_{29}H_{28}N_3O_5S_3$ ($[M+Na]^+$):616.1011, found: 616.0981.

4.1.2.7. 3-((4-((4-bromophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propyl

*3-(allyldisulfaneyl)propanoate (**9g**)*

Off-white solid, yield: 16%. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.53 (s, 1H), 8.49 (s, 1H), 7.84 (d, *J* = 12.0 Hz, 3H), 7.57 (d, *J* = 12.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 1H), 5.84-5.69 (m, 1H), 5.21-5.15 (m, 1H), 5.11-5.05 (m, 1H), 4.33-4.24 (m, 4H), 3.98 (s, 3H), 3.16 (d, *J* = 8.0 Hz, 2H), 2.67-2.61 (m, 2H), 2.51 (s, 2H), 2.20-2.14 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 172.0, 168.9, 156.5, 153.9, 153.2, 147.4, 139.5, 135.0, 131.7, 128.8, 124.4, 117.6, 115.3, 109.4, 102.5, 65.7, 61.7, 56.8, 34.5, 34.1, 28.3; LC-MS *m/z*: 563.8[M+H]$^{+}$; MS (*m/z*): HRMS(ESI) Calcd for $C_{24}H_{25}BrN_3O_4S_2$ ([M-H]$^{-}$): 562.0470(100%), found:562.0589; Calcd for $C_{24}H_{25}BrN_3O_4S_2$ ([M-H]$^{-}$): 564.0449(97.3%), found:564.0569.

*4.1.2.8. 3-((4-((4-bromophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)propyl 3-(allyldisulfaneyl)propanoate (**9h**)*

Off-white solid, yield: 20%. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.54 (s, 1H), 8.53 (s, 1H), 8.16 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H), 7.36 (t, *J* = 8.0 Hz 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.24-7.22 (m, 1H), 5.79-5.69 (m, 1H), 5.14-5.05 (m, 2H), 4.33-4.23 (m, 4H), 3.98 (s, 3H), 3.16 (d, *J* = 4.0 Hz, 2H), 2.63 (t, *J* = 4.0 Hz ,2H), 2.52-2.51 (m, 2H), 2.17-2.11 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 172.0, 166.0, 156.4, 154.0, 153.1, 149.6, 147.5, 135.0, 130.8, 128.8, 126.0, 124.4, 121.6, 121.0, 117.6, 109.4, 102.4, 65.7, 61.5, 56.8, 34.5, 34.1, 28.1; LC-MS *m/z*: 564.1[M+H]$^{+}$; MS (*m/z*): HRMS(ESI) Calcd for ([M-H]$^{-}$): 562.0470(100%), found:562.0494; Calcd for $C_{24}H_{25}BrN_3O_4S_2$ ([M-H]$^{-}$): 564.0449(97.3%), found:564.0481.

4.1.3. Synthesis of compounds (**11-14**)

Compounds **11-14** were prepared according to a similar procedure in Refs[21].

4.1.4. General procedure for the preparation of the compounds **15**.

To the corresponding H_2S donors (1.1 mmol) in DMF (10 mL) was added HATU (1.1 mmol) and triethylamine (1.2 mmol) at room temperature and the reaction was run for 20 mins. Then compounds **14** (1.0 mmol) was added, and the reaction mixture was

stirred for 24 h at room temperature. After completion of the reaction, the mixture was poured into ice water (100 mL), stirred for 30mins. The solid formed was filtered off and purified by a silica gel column chromatography to afford compound **15**.

*4.1.4.1 2-(4-(3-thioxo-3H-1, 2-dithiol-5-yl)phenoxy)-N-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)acetamide (**15a**)*

Yellow solid, yield: 16.6 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.52 (s, 1H), 10.05 (s, 1H), 8.79 (s, 1H), 8.60 (s, 1H), 8.27 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.96-7.91 (m, 3H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 4.0 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 4.95 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.4, 176.9, 166.8, 161.7, 157.9, 153.7, 147.4, 140.73, 136.5, 134.9, 129.9, 129.90, 129.6, 129.5, 129.0, 128.7, 128.1, 128.10, 126.2, 124.8, 124.6, 118.7, 116.3, 115.9, 67.5; LC-MS *m/z*: 571.1 $[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{26}H_{16}F_3N_4O_2S_3$ ($[M-H]^-$): 569.0387, found:569.0453.

4.1.4.2

*N-(4-((3-bromophenyl)amino)quinazolin-6-yl)-2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phe noxy)acetamide (**15b**)*

Yellow solid, yield: 10.0 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.55 (s, 1H), 9.90 (s, 1H), 8.77 (s, 1H), 8.57 (s, 1H), 8.17 (s, 1H), 7.97 (d, *J* = 12.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.72 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.25 (s, 1H), 7.19 (d, *J* = 8.0 Hz, 2H), 4.93 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.3, 173.9, 166.7, 161.7, 157.8, 153.7, 147.4, 141.6, 136.4, 134.8, 130.6, 129.4, 128.7, 126.3, 125.6, 124.8, 121.6, 121.3, 116.3, 116.1, 115.9, 67.5; LC-MS *m/z*: 581.0$[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{25}H_{16}BrN_4O_2S_3$ ($[M-H]^-$): 578.9619(100%), found:578.9648; Calcd for $C_{25}H_{16}BrN_4O_2S_3$ ($[M-H]^-$): 580.9598(97.3%), found:580.9629.

4.1.4.3

*N-(4-((3-chloro-4-fluorophenyl)amino)quinazolin-6-yl)-2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)acetamide (**15c**)*

Yellow solid, yield: 15.0 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.76 (s, 1H), 8.53 (s, 1H), 7.98 (d, *J* = 12.0 Hz, 2H), 7.75-7.70 (m, 3H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.19-7.16 (m, 2H), 4.79 (s, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 215.3, 172.4, 166.7, 161.6, 136.8, 134.8, 129.3, 128.6, 128.2, 124.9, 124.7, 119.5, 119.4, 116.9, 116.7, 116.2, 116.0, 113.1, 100.0, 67.5; LC-MS *m/z*: 555.0$[M+H]^+$; MS(*m/z*):HRMS(ESI) Calcd for $C_{25}H_{17}ClFN_4O_2S_3$($[M+H]^+$): 556.0654, found:556.0680.

4.1.4.4

*3-(allyldisulfaneyl)-N-(4-((3-(trifluoromethyl)phenyl)amino)quinazolin-6-yl)propanamide (**15d**)*

Off-white solid, yield: 16.0 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.37 (s, 1H), 10.02 (s, 1H), 8.76 (s, 1H), 8.57 (s, 1H), 8.27-8.20 (m, 2H), 7.82 (dd, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.41 (s, 1H), 5.90-5.80 (m, 1H), 5.19 (dd, *J* = 16.0 Hz, 2H), 3.43 (d, *J* = 8.0 Hz, 2H), 3.05 (m, 2H), 2.51-2.50 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.9, 157.8, 149.5, 147.1, 140.8, 137.3, 134.0, 129.9, 129.0, 128.0, 126.1, 126.1, 119.2, 118.4, 117.3, 116.0, 100.0, 41.5, 36.4, 33.9; LC-MS *m/z*: 465.1$[M+H]^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{21}H_{19}F_3N_4NaOS_2$ ($[M+H]^+$): 465.1031, found: 465.1007; Calcd for $C_{21}H_{19}F_3N_4NaOS_2$ ($[M+Na]^+$): 487.0850, found: 487.0836.

4.1.4.5

*3-(allyldisulfaneyl)-N-(4-((3-bromophenyl)amino)quinazolin-6-yl)propanamide (**15e**)*

Off-white solid, yield: 15.7 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.35 (s, 1H), 9.88 (s, 1H), 8.71 (s, 1H), 8.57 (d, *J* = 4.0 Hz, H), 8.17 (t, *J* = 4.0 Hz, 1H), 7.87 (d, *J* =8.0 Hz, 2H), 7.78 (dd, *J* = 4.0 Hz, 1H), 7.32-7.25 (m, 2H), 5.90-5.80 (m, 1H), 5.18 (dd, *J* =16.0 Hz, 2H), 3.43 (d, *J* = 8.0 Hz, 2H), 3.04 (t, *J* = 8.0 Hz, 2H), 2.51-2.50 (m, 2H);

^{13}C NMR (100 MHz, d_6-DMSO) δ 169.9, 157.8, 153.5, 147.1, 141.6, 137.3, 134.0, 130.7, 128.9, 126.4, 124.8, 121.6, 121.4, 119.2, 115.9, 100.0, 41.5, 36.4, 33.9; LC-MS *m/z*: 475.0[M+H]$^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{20}H_{18}BrN_4OS_2$ ([M-H]$^-$): 473.0105(100%), found:473.0166; Calcd for $C_{20}H_{18}BrN_4OS_2$ ([M-H]$^-$): 475.0085(97.3%), found:475.0153.

4.1.4.6

*3-(allyldisulfaneyl)-N-(4-((3-chloro-4-fluorophenyl)amino)quinazolin-6-yl)propanamide (**15f**)*

Off-white solid, yield: 17.0 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.35 (s, 1H), 9.90 (s, 1H), 8.71 (s, 1H), 8.54 (s, 1H), 8.13 (dd, *J* =4.0 Hz, 1H), 7.86-7.75 (m, 3H), 7.38 (s, 1H), 5.90-5.80 (m, 1H), 5.18 (dd, *J* = 16.0 Hz, 2H), 3.43 (d, *J* = 8.0 Hz, 2H), 3.04 (t, *J* = 8.0 Hz, 2H), 2.51-2.50 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 169.9, 157.8, 153.4, 152.6, 147.0, 137.3, 137.1, 134.0, 128.9, 124.3, 123.1, 119.3, 119.1, 117.0, 115.8, 112.0, 41.5, 36.4, 34.0; LC-MS *m/z*: 449.1[M+H]$^+$; MS (*m/z*): HRMS(ESI) Calcd for $C_{20}H_{17}ClFN_4OS_2$ ([M-H]$^-$):447.0516(100.0%), found:447.0608; Calcd for $C_{20}H_{17}ClFN_4OS_2$ ([M-H]$^-$):448.0550(21.6%), found:448.0573; Calcd for $C_{20}H_{17}ClFN_4OS_2$ ([M-H]$^-$):449.0487(32.0%), found:449.0534.

4.2. General Procedure for Anti-proliferation Assays

Cells were seeded in 96-well culture plates (2500−3000/well). The compounds of various concentrations were added to the plates. Cell proliferation was determined after treatment with compounds for 72 h. Cell viability was measured using the Cell Titer-Glo assay (Promega, USA) according to the manufacturer's instructions, and luminescence was measured in a multi-label reader (Envision, PerkinElmer, USA). Data were normalized to control groups (DMSO) and represented by the mean of three independent measurements with standard error of <20%. GI_{50} values were calculated using Prism 5.0 (GraphPad Software, San Diego, CA).

4.3. Western blotting analysis

H3255 cells were treated with compounds at different concentrations. Cells were then harvested, washed with ice-cold PBS and lysed in cold RIPA buffer (containing 1% PMSF). The cells were centrifuged at 16200 rpm at 4 °C for 10 min and the supernatant was collected as whole cell lysate. Protein concentration was determined by BCA method. 1 x loading buffer was added and samples were heated to 100 °C for 10mins. Proteins were separated by SDS/PAGE electrophoresis and transferred to PVDF membranes. After blocking the membranes with 5% fat-free milk, the target protein was proved with anti-phospho specific EGFR (Py1068) antibodies. Blots were developed by enhanced chemiluminescence (BD FACSCalibur).

4.4. Cell cycle analysis

H3255 and Pffeifer cells in 10% FBS/RPMI were treated with DMSO or compound **9h** (0-10μM) for 72 hours and washed with cold 1×PBS buffer. The cells were then fixed in 70% cold ethanol and incubated at -20 °C overnight. On the day of flow cytometry, cells were spun down, washed with 1×PBS buffer and stained in PI/RNase Staining Buffer (BD Bioscience) for 15 min at room temperature and moved to 4 °C until the time of analysis. Flow cytometry was performed using a FACS Calibur (BD), and results were analyzed by ModFit software.

4.5 hydrogen sulfide release evaluation

The H_2S fluorescent probe **NIR-HS** (10μM) and the target compounds (0μM, 5μM, 10μM, 50μM, 100μM and 200μM) were incubated in PBS buffer (PBS: acetone =2:3) for 3h (37°C). Subsequently, the fluorescence emission spectra (λ_{ex}=670nm, λ_{em}=723nm) were determined by an F4600 fluorescence spectrophotometer.

The H_2S fluorescent probe **NIR-HS** (10μM) and Na_2S (0μM, 2μM, 4μM, 6μM and 8μM) were incubated in PBS buffer (PBS: acetone =2:3) for 3h (37°C). Then the fluorescence emission spectra (λ_{ex}=670nm, λ_{em}=723nm) were determined by an F4600 fluorescence spectrophotometer, and the standard curve was established

according to the fluorescence intensity.

The H_2S fluorescent probe **NIR-HS** (10μM) and target compounds (20μM) were incubated in PBS buffer solution (PBS: acetone =2:3) for 0min, 5min, 30min, 60min, 90min, 120min, 150min, 180min (37°C), respectively. After that, fluorescence emission spectra (λ_{ex}=670nm, λ_{em}=723nm) were determined. According to Na_2S standard curve, the amount of H_2S released by compounds was converted.

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References

[1] G.I. Solyanik, Quinazoline compounds for antitumor treatment, Experimental oncology, 41 (2019) 3-6.

[2] E.-d. Li, Q. Lin, Y.-q. Meng, L.-y. Zhang, P.-p. Song, N. Li, J.-c. Xin, P. Yang, C.-n. Bao, D.-q. Zhang, Y. Zhang, J.-k. Wang, Q.-r. Zhang, H.-m. Liu, 2,4-Disubstituted quinazolines targeting breast cancer cells via EGFR-PI3K, European Journal of Medicinal Chemistry, 172 (2019) 36-47.

[3] H.S.A. Al-Salem, G.H. Hegazy, K.E.H. El-Taher, S.M. El-Messery, A.M. Al-Obaid, H.I. El-Subbagh, Synthesis, anticonvulsant activity and molecular modeling study of some new hydrazinecarbothioamide, benzenesulfonohydrazide, and phenacylacetohydrazide analogues of 4(3H)-quinazolinone, Bioorganic & Medicinal Chemistry Letters, 25 (2015) 1490-1499.

[4] M.A. Pradeep, N.R. Kumar, D.K. Swaroop, N.S. Reddy, K. Sirisha, C.G. Kumar, N.J. Babu, T. Ganapathi, B. Narsaiah, Design and Synthesis of Novel Pyrimidine/Hexahydroquinazoline-Fused Pyrazolo 3,4-b Pyridine Derivatives, Their Biological Evaluation and Docking Studies(#), Chemistryselect, 4 (2019) 138-144.

[5] M. Yu V, A. O M, N. I S, B. G G, K. S I, Directed Search of Anti-inflammatory Agents among (3H-Quinazoline-4-ylidene)hydrazides of N-protected Amino acids and their Heterocyclization Products, Anti-inflammatory & anti-allergy agents in medicinal chemistry, (2019).

[6] R. Neelarapu, J.R. Maignan, C.L. Lichorowic, A. Monastyrskyi, T.S. Mutka, A.N. LaCrue, L.D. Blake, D. Casandra, S. Mashkouri, J.N. Burrows, P.A. Willis, D.E. Kyle, R. Manetsch, Design and Synthesis of Orally Bioavailable Piperazine Substituted 4(1H)-Quinolones with Potent Antimalarial Activity: Structure-Activity and Structure-Property Relationship Studies, Journal of Medicinal Chemistry, 61 (2018) 1450-1473.

[7] L.M. Antypenko, S.I. Kovalenko, O.V. Karpenko, A.M. Katsev, V.P. Novikov, N.S. Fedyunina, 1-R-2-(1,2,4 Triazolo 1,5-c quinazoline-2-ylthio) etanon(ol) s: Synthesis, Bioluminescence Inhibition, Molecular Docking Studies, Antibacterial and Antifungal Activities, Current Computer-Aided Drug

Design, 12 (2016) 29-41.

[8] M.U. Rahman, A. Rathore, A.A. Siddiqui, G. Parveen, M.S. Yar, Synthesis and characterization of quinazoline derivatives: search for hybrid molecule as diuretic and antihypertensive agents, Journal of Enzyme Inhibition and Medicinal Chemistry, 29 (2014) 733-743.

[9] R.P. Modh, E. De Clercq, C. Pannecouque, K.H. Chikhalia, Design, synthesis, antimicrobial activity and anti-HIV activity evaluation of novel hybrid quinazoline-triazine derivatives, Journal of Enzyme Inhibition and Medicinal Chemistry, 29 (2014) 100-108.

[10] D. Das, L. Xie, J. Wang, X. Xu, Z. Zhang, J. Shi, X. Le, J. Hong, Discovery of new quinazoline derivatives as irreversible dual EGFR/HER2 inhibitors and their anticancer activities - Part 1, Bioorganic & Medicinal Chemistry Letters, 29 (2019) 591-596.

[11] S. Lee, J. Kim, K.B. Duggirala, A. Go, I. Shin, B.C. Cho, G. Choi, C.H. Chae, K. Lee, Allosteric Inhibitor TREA-0236 Containing Non-hydrolysable Quinazoline-4-one for EGFR T790M/C797S Mutants Inhibition, Bulletin of the Korean Chemical Society, 39 (2018) 895-898.

[12] M.M. Ghorab, M.S. Alsaid, A.M. Soliman, Dual EGFR/HER2 inhibitors and apoptosis inducers: New benzo g quinazoline derivatives bearing benzenesulfonamide as anticancer and radiosensitizers, Bioorganic Chemistry, 80 (2018) 611-620.

[13] C. Wang, S. Xu, L. Peng, B. Zhang, H. Zhang, Y. Hu, P. Zheng, W. Zhu, Design, synthesis and biological evaluation of novel 4-anlinoquinazoline derivatives as EGFR inhibitors with the potential to inhibit the gefitinib-resistant nonsmall cell lung cancers, Journal of Enzyme Inhibition and Medicinal Chemistry, 34 (2019) 204-218.

[14] P. Pathak, P.K. Shukla, V. Kumar, A. Kumar, A. Verma, Quinazoline clubbed 1,3,5-triazine derivatives as VEGFR2 kinase inhibitors: design, synthesis, docking, in vitro cytotoxicity and in ovo antiangiogenic activity, Inflammopharmacology, 26 (2018) 1441-1453.

[15] S.J. Buggana, M.C. Paturi, H. Perka, G.D. Reddy, V.V.S.R. Prasad, Novel 2,4-disubstituted quinazolines as cytotoxic agents and JAK2 inhibitors: Synthesis, in vitro evaluation and molecular dynamics studies, Computational Biology and Chemistry, 79 (2019) 110-118.

[16] J. Chen, Z. Sang, Y. Jiang, C. Yang, L. He, Design, synthesis, and biological evaluation of quinazoline derivatives as dual HDAC1 and HDAC6 inhibitors for the treatment of cancer, Chemical Biology & Drug Design, 93 (2019) 232-241.

[17] S.-M. Yang, D.J. Urban, M. Yoshioka, J.W. Strovel, S. Fletcher, A.Q. Wang, X. Xu, P. Shah, X. Hu, M.D. Hall, A. Jadhav, D.J. Maloney, Discovery and lead identification of quinazoline-based BRD4 inhibitors, Bioorganic & Medicinal Chemistry Letters, 28 (2018) 3483-3488.

[18] X. Hu, H. Zhao, Y. Wang, Z. Liu, B. Feng, C. Tang, Synthesis and biological evaluation of novel 5,6-dihydropyrimido 4,5-f quinazoline derivatives as potent CDK2 inhibitors, Bioorganic & Medicinal Chemistry Letters, 28 (2018) 3385-3390.

[19] J. Zhou, M. Ji, H. Yao, R. Cao, H. Zhao, X. Wang, X. Chen, B. Xu, Discovery of quinazoline-2,4(1H,3H)-dione derivatives as novel PARP-1/2 inhibitors: design, synthesis and their antitumor activity, Organic & Biomolecular Chemistry, 16 (2018) 3189-3202.

[20] S. Patel, A. Motta, P. Flaherty, A. Bhatt, T. Wright, J. Cavanaugh, 4,6-Disubstituted quinazoline derivatives as inhibitors of the MEK5/ERK5 pathway, Abstracts of Papers of the American Chemical Society, 256 (2018).

[21] Y.-G. Zheng, J. Su, C.-Y. Gao, P. Jiang, L. An, Y.-S. Xue, J. Gao, Y. Liu, Design, synthesis, and biological evaluation of novel 4-anilinoquinazoline derivatives bearing amino acid moiety as potential EGFR kinase inhibitors, European Journal of Medicinal Chemistry, 130 (2017) 393-405.

[22] M. Zheng, Y. Zheng, Y. Xue, Y. Liu, L. An, L. Zhang, M. Ji, B. Xue, X. Wu, X. Gong, N. Gu, X. Zhan, Synthesis and Quantum Chemical Studies of New 4-aminoquinazoline Derivatives as Aurora A/B Kinase Inhibitors, Chemical Biology & Drug Design, 81 (2013) 399-407.

[23] X. Wu, M. Li, Y. Qu, W. Tang, Y. Zheng, J. Lian, M. Ji, L. Xu, Design and synthesis of novel Gefitinib analogues with improved anti-tumor activity, Bioorganic & Medicinal Chemistry, 18 (2010) 3812-3822.

[24] C.-M. Park, Y. Zhao, Z. Zhu, A. Pacheco, B. Peng, N.O. Devarie-Baez, P. Bagdon, H. Zhang, M. Xian, Synthesis and evaluation of phosphorodithioate-based hydrogen sulfide donors, Molecular Biosystems, 9 (2013) 2430-2434.

[25] Y. Ma, Z. Yan, X. Deng, J. Guo, J. Hu, Y. Yu, F. Jiao, Anticancer effect of exogenous hydrogen sulfide in cisplatin-resistant A549/DDP cells, Oncology Reports, 39 (2018) 2969-2977.

[26] S. Lu, Y. Gao, X. Huang, X. Wang, GYY4137, a hydrogen sulfide (H2S) donor, shows potent anti- hepatocellular carcinoma activity through blocking the STAT3 pathway, International Journal of Oncology, 44 (2014) 1259-1267.

[27] M. Ciocci, E. Iorio, F. Carotenuto, H.A. Khashoggi, F. Nanni, S. Melino, H2S-releasing nanoemulsions: a new formulation to inhibit tumor cells proliferation and improve tissue repair, Oncotarget, 7 (2016) 84338-84358.

[28] S. Pu, X. Wu, X. Yang, Y. Zhang, Y. Dai, Y. Zhang, X. Wu, Y. Liu, X. Cui, H. Jin, J. Cao, R. Li, J. Cai, Q. Cao, L. Hu, Y. Gao, The Therapeutic Role of Xenobiotic Nuclear Receptors Against Metabolic Syndrome, Curr Drug Metab, 20 (2019) 15-22.

[29] Z.W. Lee, J. Zhou, C.-S. Chen, Y. Zhao, C.-H. Tan, L. Li, P.K. Moore, L.-W. Deng, The Slow-Releasing Hydrogen Sulfide Donor, GYY4137, Exhibits Novel Anti-Cancer Effects In Vitro and In Vivo, Plos One, 6 (2011).

[30] X. Hu, R. Jiao, H. Li, X. Wang, H. Lyu, X. Gao, F. Xu, Z. Li, H. Hua, D. Li, Antiproliferative hydrogen sulfide releasing evodiamine derivatives and their apoptosis inducing properties, European Journal of Medicinal Chemistry, 151 (2018) 376-388.

[31] F. Xu, X. Gao, H. Li, S. Xu, X. Li, X. Hu, Z. Li, J. Xu, H. Hua, D. Li, Hydrogen sulfide releasing enmein-type diterpenoid derivatives as apoptosis inducers through mitochondria-related pathways, Bioorganic Chemistry, 82 (2019) 192-203.

[32] R. Kodela, M. Chattopadhyay, K. Kashfi, Synthesis and biological activity of NOSH-naproxen (AVT-219) and NOSH-sulindac (AVT-18A) as potent anti-inflammatory agents with chemotherapeutic potential, Medchemcomm, 4 (2013) 1472-1481.

[33] R. Kodela, M. Chattopadhyay, K. Kashfi, NOSH-Aspirin: A Novel Nitric Oxide-Hydrogen Sulfide-Releasing Hybrid: A New Class of Anti-inflammatory Pharmaceuticals, Acs Medicinal Chemistry Letters, 3 (2012) 257-262.

[34] S. Wu, J. Fu, Y. Dong, Q. Yi, D. Lu, W. Wang, Y. Qi, R. Yu, X. Zhou, GOLPH3 promotes glioma progression via facilitating JAK2-STAT3 pathway activation, Journal of neuro-oncology, 139 (2018) 269-279.

[35] X.P. Zhou, S. Xie, S.S. Wu, Y.H. Qi, Z.H. Wang, H. Zhang, D. Lu, X. Wang, Y. Dong, G.Z. Liu, D.X. Yang, Q. Shi, W.B. Bian, R.T. Yu, Golgi phosphoprotein 3 promotes glioma progression via inhibiting Rab5-mediated endocytosis and degradation of epidermal growth factor receptor, Neuro-Oncology, 19 (2017) 1628-1639.

[36] J.G. Paez, P.A. Janne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F.J. Kaye, N. Lindeman, T.J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M.J. Eck, W.R. Sellers, B.E. Johnson, M.

Meyerson, EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy, Science, 304 (2004) 1497-1500.

[37] X.X. Li, A.L. Wang, K.L. Yu, Z.P. Qi, C. Chen, W.C. Wang, C. Hu, H. Wu, J.X. Wu, Z. Zhao, J. Liu, F.M. Zou, L. Wang, B.L. Wang, W. Wang, S.C. Zhang, J. Liu, Q.S. Liu, Discovery of (R)-1-(3-(4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo 3,4-d pyrimidin-1-yl)p iperidin-1-yl)-2-(dimethylamino)ethanone (CHMFL-FLT3-122) as a Potent and Orally Available FLT3 Kinase Inhibitor for FLT3-ITD Positive Acute Myeloid Leukemia, Journal of Medicinal Chemistry, 58 (2015) 9625-9638.

[38] J.S. Melnick, J. Janes, S. Kim, J.Y. Chang, D.G. Sipes, D. Gunderson, L. Jarnes, J.T. Matzen, M.E. Garcia, T.L. Hood, R. Beigi, G. Xia, R.A. Harig, H. Asatryan, S.F. Yan, Y.Y. Zhou, X.J. Gu, A. Saadat, V. Zhou, F.J. King, C.M. Shaw, A.I. Su, R. Downs, N.S. Gray, P.G. Schultz, M. Warmuth, J.S. Caldwell, An efficient rapid system for profiling the cellular activities of molecular libraries, Proceedings of the National Academy of Sciences of the United States of America, 103 (2006) 3153-3158.

[39] S. La Monica, D. Cretella, M. Bonelli, C. Fumarola, A. Cavazzoni, G. Digiacomo, L. Flammini, E. Barocelli, R. Minari, N. Naldi, P.G. Petronini, M. Tiseo, R. Alfieri, Trastuzumab emtansine delays and overcomes resistance to the third-generation EGFR-TKI osimertinib in NSCLC EGFR mutated cell lines, Journal of Experimental & Clinical Cancer Research, 36 (2017).

[40] N.J. Zhao, P.C. Liang, X.Y. Zhuo, C.L. Su, X.M. Zong, B.N. Guo, D. Han, X.L. Yan, S.Q. Hu, Q.G. Zhang, X. Tie, After Treatment with Methylene Blue is Effective against Delayed Encephalopathy after Acute Carbon Monoxide Poisoning, Basic & clinical pharmacology & toxicology, 122 (2018) 470-480.

[41] C. Pham-Danis, S. Gehrke, E. Danis, A.I. Rozhok, M.W. Daniels, D.X. Gao, C. Collins, J.T. Di Paola, A. D'Alessandro, J. DeGregori, Urea Cycle Sustains Cellular Energetics upon EGFR Inhibition in EGFR-Mutant NSCLC, Molecular Cancer Research, 17 (2019) 1351-1364.

[42] Y.L. Zhang, L. Chen, H.J. Xu, X.B. Li, L.J. Zhao, W. Wang, B.L. Li, X.Q. Zhang, 6,7-Dimorpholinoalkoxy quinazoline derivatives as potent EGFR inhibitors with enhanced antiproliferative activities against tumor cells, European Journal of Medicinal Chemistry, 147 (2018) 77-89.

[43] L. Zhang, X.E. Zheng, F. Zou, Y.G. Shang, W.Q. Meng, E. Lai, Z.C. Xu, Y. Liu, J. Zhao, A highly selective and sensitive near-infrared fluorescent probe for imaging of hydrogen sulphide in living cells and mice, Scientific Reports, 6 (2016).

[44] N. Lougiakis, A. Papapetropoulos, E. Gikas, S. Toumpas, P. Efentakis, R. Wedmann, A. Zoga, Z.M. Zhou, E.K. Iliodromitis, A.L. Skaltsounis, M.R. Filipovic, N. Pouli, P. Marakos, I. Andreadou, Synthesis and Pharmacological Evaluation of Novel Adenine-Hydrogen Sulfide Slow Release Hybrids Designed as Multitarget Cardioprotective Agents, Journal of Medicinal Chemistry, 59 (2016) 1776-1790.

Highlights

- We devised and synthesized a series of quinazoline derivatives bearing H_2S donors.
- The target compounds exhibits inhibiting activities against TEL-EGFR-L858R-BaF3.
- Most compounds displayed anti-proliferative activities against H3255 cells.
- Compound **9h** exhibits lower toxic effect on other tumor cells and HUVEC cells.
- Compound **9h** inhibits the phosphorylation of EGFR in H3255 cells.
- compound **9h** induces cell cycle arrest in G0-G1 phase.
- The H_2S release of compound **9h** is more and faster than other compounds.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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