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Research paper

Synthesis of triazoloquinazolinone based compounds as tubulin polymerization inhibitors and vascular disrupting agents

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ABSTRACT

A series of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones designed as conformationally restricted CA-4 analogues, were tested for their tubulin polymerization and growth inhibitory activities. The 3-hydroxy-4-methoxy derivatives **11d** and **12d** are potent inhibitors of tubulin assembly but only the *N*-methylated amid counterpart **12d** possesses potent anticancer activity in a large panel of cancer cell lines. Upon treatment with compound **12d**, remarkable cell shape changes as cell migration and tube formation were elicited in HUVECs, consistent with vasculature damaging activity.

1. Introduction

Microtubules provide an important framework supporting cellular morphology in interphase, and they are essential in cell division as the key component of the mitotic spindle. Consequently, the microtubule has become an important target for the design of new antimitotic anticancer agents. The antimitotic agents currently in clinical use include vinca alkaloids [1], which inhibit microtubule polymerization, and toxoids [2], which promote microtubule assembly, but the development of drug resistance limits its usefulness [3–5]. Combretastatin A-4 (CA-4, Fig. 1) is another well-known antimitotic agent, derived from the African bush willow *Combretum caffrum* and has been first described over 20 years ago [6]. It is a strong tubulin depolymerizing agent (TDA) and therefore inhibits tumor growth and has antivascular effects [7]. Its prodrug (disodium salt water-soluble phosphate derivative, CA-4P) has now entered clinical trials for both solid and liquid tumors [8]. The encouraging antivascular and antitumor profile of CA-4 has contributed greatly to the current interest in the design and synthesis of several CA-4 analogues [9].

Through SAR studies, it has been established that the cis orientation of both phenyl groups is an essential requirement for efficient tubulin affinity, forcing the two aromatic rings to stay within appropriate angles and distances in the colchicine binding site (Fig. 2A). In fact, the cis double bond of CA-4 or analogues easily undergoes isomerization, leading to trans isomers that display dramatically decreased inhibitions of cancer cell growth and tubulin assembly. In order to stabilize the active configuration, numerous teams have synthesized a wide range of various cis-restricted analogs of CA-4 by replacement of the double bond with other rigid linkers [10] or different cyclic moieties [11], or by introducing other rings [12].

Quinazolinone skeleton, which is considered as a privileged structure [13], has been demonstrated to conduct to antimitotic agents, exerting their antitumor activity through inhibition of the DNA repair enzyme system or dysregulation of cell cycle

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progression of cancer cells [14,15]. Among the various classes of antimitotics, quinazolin-4-ones form an important component of pharmacologically active compounds as they are associated with inhibitory effects on tubulin polymerization and the anticancer activities of 2-styrylquinazolin-4-ones [16], 2-aryl and 2,3-dihydro-2-arylquinazolin-4-ones [14,15,17] and tetrahydropyrido[2,1-*b*] quinazolin-10-ones [18] are well established. These considerations led us to design a new series of conformationally restricted CA-4 analogues by replacing the cis-olefinic bond by a quinazolin-4-one based heterocycle.

In the past recent years, a number of 1-substituted-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones have revealed antitoxoplasmosis [19], H1-antihistaminic [20], antimicrobial [21] anticonvulsant [22] and PDE4 inhibitory [23] properties. As the two aromatic rings of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one derivatives adopt also a conformation in which they are not coplanar (Fig. 2B), the structural similarity between combretastatins and these tricyclic compounds can be expected to lead to an activity on the colchicine site of tubulin in the case of conveniently substituted derivatives. Moreover, such a structure will avoid inactivation resulting from cis to trans isomerization of the double bond of combretastatin derivatives. We therefore decided to investigate the synthesis of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one analogues of CA-4 and to study their biological efficiency toward tubulin polymerization, their cytotoxicities toward various cancer cell lines as well as their antivascular effects.

2. Results and discussion

2.1. Chemistry

The synthesis of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one derivatives is well described in the literature and followed the general methods depicted in Scheme 1. Methyl anthranilate **1** reacted with thiophosgene to provide the corresponding thioisocyanate **2** which on refluxing in toluene with benzylamine or methylamine 40% aqueous solution yielded the desired 3-substituted-2-sulfanylquinazolin-4-ones **3** and **4** via the thiourea intermediates in good yields. Nucleophilic displacement of sulfanyl group in refluxing ethanol with hydrazine hydrate in large excess yielded the desired 2-hydrazinoquinazolin-4-ones **5** and **6** with respectively, 86% and 89% yield. One of the useful methods for the preparation of fused 1,2,4-triazoles is based on oxidative cyclization of the fused heterocyclic hydrazones with iron(III) chloride as oxidant [24]. The hydrazones **7a-g** and **8a-g** were then prepared by condensation of the corresponding hydrazines with equimolar amount of benzaldehyde derivatives in refluxing methanol and the presence of a catalytic amount of acetic acid. Treatment of the hydrazones with hot ethanolic iron(III) chloride solution resulted in an *in situ* 1,5-electrocyclization to the angularly annelated 1,2,4-triazolo[4,3-*a*]quinazolin-5-ones **9a-g** and **10a-g** in very good yields. Finally, decomposition of ammonium formate with catalytic amount of Pd/C in refluxing ethanol conducted to the hydrogenolysis of the benzylheteroatom bonds but also to the reduction of the nitro group to provide the N- and O-deprotected derivatives **11a-e** and **12d-g**. For compounds **9f** and **9g**, these conditions did not allow hydrogenolysis of the N-benzyl protection, even in the presence of a large amount of ammonium formate and Pd/C; only the benzyloxy and nitro groups were deprotected and reduced providing derivatives **11f** and **11g**.

2.2. In vitro tubulin polymerization and antiproliferative assays

To characterize the possible interaction with the microtubule system of this novel series of triazoloquinazolinone derivatives, compounds **10a-c**, **11a-e** and **12d-g** were evaluated for their *in vitro* inhibition of tubulin polymerization (Table 1). Namely, some recent reports suggest that nonlinear relationship between antiproliferative activity and the effect on tubulin polymerization of described inhibitors, as combretastatin A-4, may occur, where highly cytotoxic compounds are not necessarily potent inhibitors of tubulin polymerization and *vice versa* [25,26].

In this assembly assay, only compounds **11d** and **12d** displayed potent inhibitory activities with IC_{50} values of 4.26 and 0.15 μM respectively whereas the others seemed to be inactive as inhibitors of tubulin polymerization and did not inhibit tubulin assembly at concentrations as high as 10 μM. These findings indicated that the 3-hydroxy-4-methoxyphenyl was crucial moiety for inhibition of tubulin polymerization and was preferential partial structure seen with many combretastatin derivatives. Interestingly, introduction of a 3,4,5-trimethoxyphenyl group in **9c** and **11c**, a typical pharmacophore found in many inhibitors of tubulin polymerization [27] led to a total loss of inhibitory activity. Furthermore, quite other variations of substituents within the aryl moiety resulted in inactive compounds at concentrations as high as 10 μM. The unsubstituted derivative **11d** was less potent than its counterpart **12d** whose substitution with a methyl group at the *N*-4 position of the triazoloquinazolinone seemed to be most tolerant for tubulin polymerization inhibitory effect. Antiproliferative effects of compounds **10a-c**, **11a-e** and **12d-g** were initially evaluated at a high single dose (10 μM) on human colon adenocarcinoma HT29 cell line and the representative results are summarized in Table 1. Percentages of cell proliferation inhibition of tested compounds are consistent with their antitubulin potencies. Indeed, the best antitubulin agent **12d** showed significant antiproliferative activity (75%) whereas its unsubstituted counterpart **11d** displayed weak

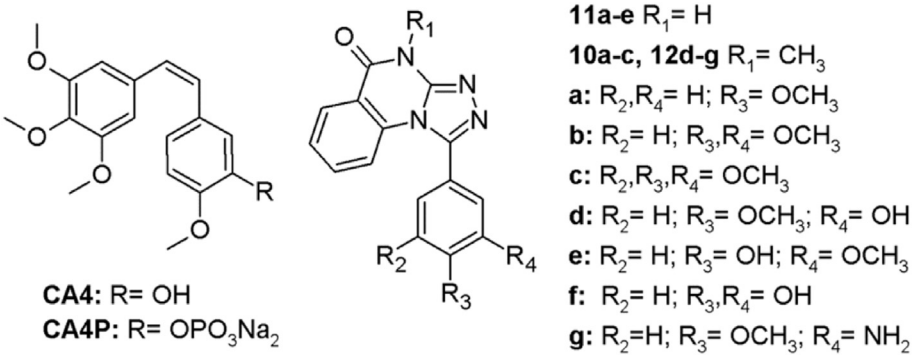


Fig. 1. Structures of reference and synthesized compounds.

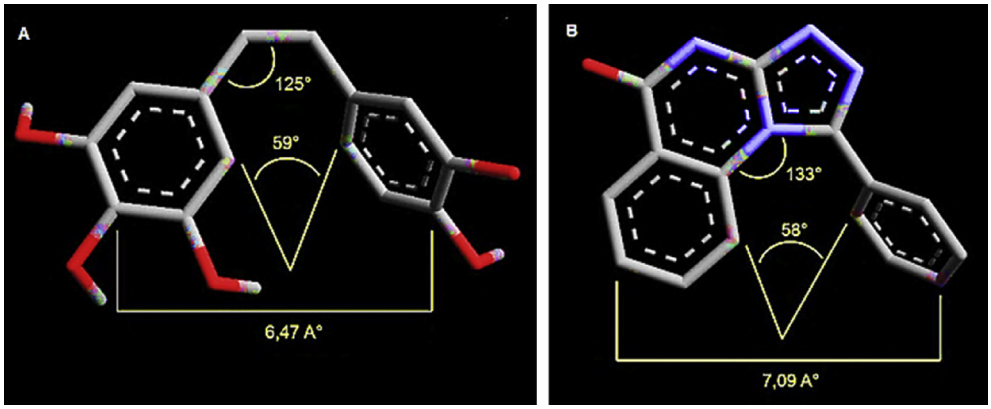
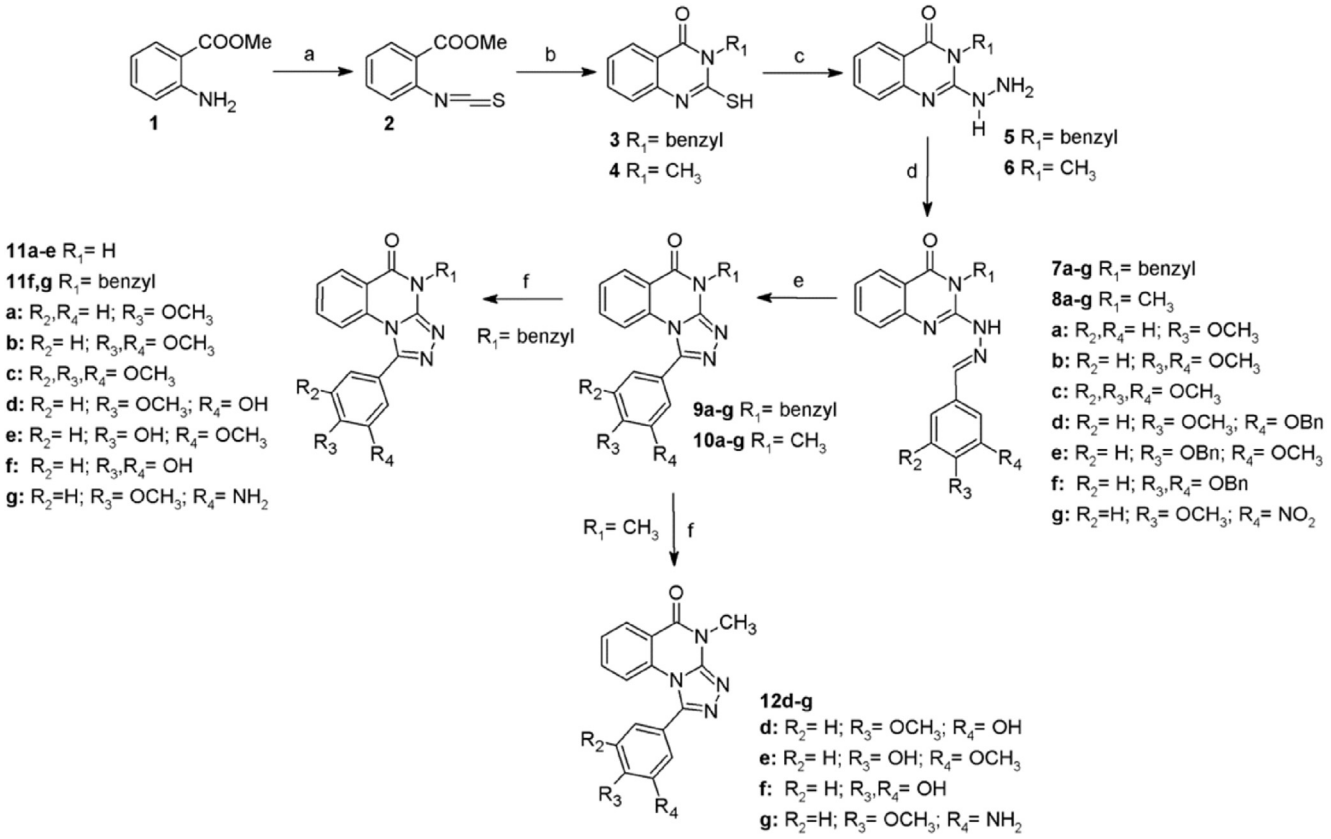


Fig. 2. Comparison of angle and distance measurements between CA-4 (A) and 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one (B).



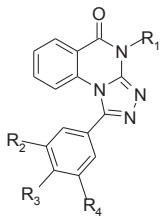
[a]Reagents and conditions: a) thiophosgene, CH_2Cl_2/water, r.t., 12h; b) methylamine 40% aqueous solution or benzylamine, toluene, reflux, 12h; c) hydrazine hydrate, ethanol, reflux, 12h; d) benzaldehyde derivative, methanol, acetic acid, reflux, 12h; e) $FeCl_3$ aqueous solution, ethanol, reflux, 2h; f) ammonium formate, Pd/C, ethanol, reflux, 12h.

Scheme 1. Synthesis of target compounds.

activities in both antitubulin and cell proliferation assays. Surprisingly, the 3-amino-4-methoxy derivative **12g** which did not inhibit tubulin assembly at concentrations as high as 10 μM, was highly cytotoxic with 95% of HT29 cell proliferation inhibition. The discrepancy between cytotoxicity and antitubulin activity was observed in several compounds in which the replacement of the phenolic OH of combretastatin derivatives by an NH_2 group is a strategy widely employed to provide more potent cytotoxic compounds. This has been noticed in various classes of antimitotic agents [28]. Since strong cytotoxicity was observed in the amino compound with weaker antitubulin activity such as **12g**, there might be another mechanism for exertion of cytotoxicity. The amino group in the 3-position plays no important role in the tubulin binding as the 3-hydroxyl group of CA-4 does [29] but it seems to be important for the strong cytotoxicity caused by the other mechanism.

Compounds **12d** and **12g** were selected by the National Cancer Institute (NCI) for screening against 60 human tumor cell lines [30].

Table 1
Structures, inhibition of tubulin polymerization and *in vitro* proliferation inhibition percentage against human colon adenocarcinoma HT29 cell line of compounds **10a-c**, **11a-e** and **12d-g**.



Compd	R_1	R_2	R_3	R_4	Inhibition[a]	IC_{50} TPI (μM)[b]
10a	CH_3	H	OCH_3	H	2%	>10
10b	CH_3	H	OCH_3	OCH_3	1%	>10
10c	CH_3	OCH_3	OCH_3	OCH_3	0%	>10
12d	CH_3	H	OCH_3	OH	71%	0.15
12e	CH_3	H	OH	OCH_3	0%	>10
12f	CH_3	H	OH	OH	0%	>10
12g	CH_3	H	OCH_3	NH_2	95%	>10
11a	H	H	OCH_3	H	0%	>10
11b	H	H	OCH_3	OCH_3	0%	>10
11c	H	OCH_3	OCH_3	OCH_3	2%	>10
11d	H	H	OCH_3	OH	10%	4.26
11e	H	H	OH	OCH_3	0%	>10

[a] % of inhibition at 10 μM inhibiting HT29 cell proliferation relative to untreated controls after 72 h of drug exposure.
[b] Inhibition of tubulin polymerization.

They were tested initially at a high single dose (10 μM) in the full 60-cell panel and have progressed to the 5-dose screen in order to evaluate their GI_{50} values (Table 2). Cytotoxic effects were registered for compound **12d** on HL-60(TB) (leukemia), NCI-H522 (NSCL cancer), MDA-MD-435 (melanoma) and OVCAR-3 (ovarian cancer) and for compound **12g** on HL-60(TB) (leukemia), COLO 205 (colon cancer) and MDA-MD-435 (melanoma). These interesting growth inhibition potentials were then confirmed *in vitro* for compounds **12d** and **12g** which displayed GI_{50} values ranging from 23 to 125 nM on various cancer cell lines (Table 2). However, some of cancer cell lines as renal (A498, CAKI-1), prostate (PC-3, DU-145), breast (MDA-MB-468) or NSCL (A549/ATCC) cancers, are less sensitive with GI_{50} values in the micromolar range. Interestingly, compound **12g** exhibited potent antiproliferative activity against HT29 and COLO 205 cells (GI_{50} of respectively 0.043 and 0.047 μM), which are highly resistant to most of tubulin polymerization inhibitors with a 3-hydroxy-4-methoxy substitution [36] as for CA-4 (GI_{50} of respectively 0.118 and 0.221 μM) or compound **12d** (GI_{50} of respectively 4.31 and 3.82 μM). One hypothesis for the CA-4 resistance in HT29 is significant glucuronidation of CA-4 by glucuronosyl transferase. It has been reported that high glucuronosyl transferase activity is observed in colorectal cancer cells and contributes to resistance to drugs such as mycophenolic acid [37]. A mean graph midpoints (MG-MID) was calculated for each derivative selected for GI_{50} calculation, giving an average activity parameter over all cell lines for compounds **12d** and **12g**. These data indicated that compounds **12d**, which acted as tubulin polymerization inhibitor, is slightly more active than its amino counterpart **12g** and deserve further investigation.

2.3. Cell invasion and endothelial tube formation assays

Besides the ability to inhibit tumor cell proliferation, microtubule-targeting drugs have also been shown to have activity against the vasculature in tumors by inhibition of endothelial cell migration and capillary tube formation [38,39]. Considering that tube formation and invasion are highly relevant properties in the process of tumor vasculature, we investigated the effects of compound **12d** at several concentrations (0.01, 0.1 and 1 μM) on the invasiveness of human umbilical endothelial cells (HUVEC) using a Boyden chamber assay. Matrigel® was applied to the filter membrane, and the number of HUVEC that penetrated the Matrigel® and membrane was quantified. As shown in Fig. 3, compound **12d** inhibited the invasiveness of HUVEC in a dose-dependent manner. Treatment with compound **12d** at 0.01, 0.1 and 1 μM inhibited invasion by 12%, 36%, and 89%, respectively.

For comparison, we studied the effects of antiangiogenic inhibitor cediranib (VEGFR, PDGFR and c-Kit inhibitor), as the reference substance, in the same experimental conditions. Compound **12d** treatment displayed similar effects, with a more pronounced inhibition of cell invasion at the concentration of 1 μM at which cediranib is less effective (inhibition of 74%).

We also tested the effects of compound **12d** on tubules formation assay, a well-known *in vitro* angiogenesis test. The antiangiogenic effect was evaluated on HUVEC by treating with the reference compound cediranib and compound **12d**. HUVEC were seeded on Geltrex® in order to imitate *in vivo* HUVEC and treated with several concentrations of cediranib or of compound **12d** (0.01, 0.1 and 1 μM) (Fig. 4). Geltrex® is an extracellular matrix, rich in pro-angiogenic factors that stimulate single endothelial cells to assume an extended shape. The overall effect results in a reticulum similar to a capillary network and offers an *in vitro* model of angiogenesis [40]. Compound **12d** was able to disrupt formed capillaries in a dose-dependent manner, after 6 h of treatment, with lower concentrations than those of the reference compound, cediranib.

To evaluate if the inhibition of cell invasion and tube formation were due to a cytotoxic action of compound **12d**, we analyzed cell proliferation of HUVEC by the MTS assay. Tumor vasculature inhibitory effects are often observed at antimitotic agent concentrations that may be 100-fold lower than those required to produce cell toxicity. For example, inhibition of endothelial cell migration and tube formation by CA-4 occurred at concentrations that were 8- to 16-fold lower than those that inhibited endothelial cell proliferation [41]. We observed similar results, although less pronounced, with compound **12d** which inhibits HUVEC proliferation with IC_{50} value of 2.48 μM after 72 h of treatment (Fig. 5) while it inhibits endothelial cell invasion and tube formation at much lower concentrations. These results showed that the inhibitory effects of cell invasion and endothelial tube formation, observed at a 1 μM concentration of test compound, were not toxic for HUVEC even after 72 h of treatment.

2.4. Modeling studies (Computational structure-based insights)

An *in silico* analysis, on the colchicine binding site of tubulin, aimed at study the positive impact of *N*-methylated amide group for tubulin activity of compound **12d** in comparison with the unsubstituted derivative **11d**. For this purpose, we used a structure-based method to examine their docking into a recent crystallographic structure of tubulin-colchicine complex recorded as 4O2B entry [42] in PDB and displaying a much higher resolution than the previous 1SA0 pdb entry [43]. First we verified that the docking solutions of colchicine into binding site converged consistently towards a unique binding mode within a 1 Å RMSd in comparison with the co-crystallized colchicine (Fig. 6a). Thus colchicine fits by hydrophobic contacts rather with the β-2B chain than the α-1B chain of tubulin. Emphasized by the composite intermolecular van der Walls and hydrogen bonding scores, the docking of colchicine is driven by a shape complementarity whereas the triazoloquinazolinone series allows hydrogen bonding between its

Table 2
Results of the *in vitro* human cancer cell growth inhibition[a] for compounds **12d** and **12g**.

Cell type	Compound	**12d**		**12g**		CA-4[a]
	Cell line	GI%[b] (10^{-5} M)	GI_{50}[c] (μM)	GI%[b] (10^{-5} M)	GI_{50}[c] (μM)	GI_{50}[c] (μM)
Leukemia	HL-60(TB)	100[e,f]	0.064	100[e,g]	N.D.[d]	0.002
	K-562	92	0.040	92	0.039	0.002
	SR	95	0.031	89	0.034	0.003
Non Small Cell Lung	A549/ATCC	82	1.84	72	1.47	0.016
(NSCL) cancer	HOP-62	90	0.048	83	0.048	0.002
	NCI-H522	100[e,h]	0.031	91	0.031	0.002
Colon cancer	COLO 205	83	3.82	100[e,i]	0.047	0.221[j]
	HCT-116	86	0.057	76	0.057	0.003
	HCT-15	81	0.052	78	0.050	0.004
	HT29	81	4.31	95	0.043	0.118
Central nervous system	SF-295	93	1.99	98	0.050	0.004
(CNS) cancer	SNB-75	88	0.041	81	N.D.[d]	0.008
	U251	82	0.125	81	1.63	0.004
Melanoma	MDA-MB-435	100[e,k]	0.023	100[e,l]	0.023	0.003[m]
	SK-MEL-5	75	0.059	86	0.060	0.003
Ovarian cancer	OVCAR-3	100[e,n]	0.045	92	0.047	0.002
	NCI/ADR-RES	85	0.062	83	0.065	N.D.[d]
Renal cancer	A498	66	6.42	80	2.84	0.006
	CAKI-1	66	1.22	65	1.95	0.025
Prostate cancer	PC-3	73	1.43	67	2.65	0.001
	DU-145	81	2.36	79	3.09	0.0005[o]
Breast cancer	MCF7	85	0.048	85	0.043	0.018[p]
	MDA-MB-468	70	4.33	89	2.33	N.D.[d]
MG-MID[q] (μM)			0.775		0.975	0.008

[a] Data obtained from NCI's *in vitro* 60 cell screening [30–32].
[b] Data obtained from NCI's *in vitro* disease-oriented human tumor cell screen at 10 μM concentration.
[c] GI_{50} is the molar concentration of synthetic compound causing 50% growth inhibition of tumor cells.
[d] Not determined.
[e] Cytotoxic effect.
[f] Cell growth percent: − 19%.
[g] Cell growth percent: − 11%.
[h] Cell growth percent: − 5%.
[i] Cell growth percent: − 18%.
[j] Data obtained from Ref. [6].
[k] Cell growth percent: − 62%.
[l] Cell growth percent: − 44%.
[m] Data obtained from Ref. [33].
[n] Cell growth percent: − 16%.
[o] Data obtained from Ref. [34].
[p] Data obtained from Ref. [35].
[q] Average activity parameter over all cell lines for the tested compounds.

acceptor hydroxyl group and Lys352 of β-tubulin particularly. The docking of compound **12d** in 4O2B pdb aporeceptor converged also towards a unique binding mode but quite different from the colchicine binding (Fig. 6b). The plane of its central heterocycle shifts by 60° from the average plane of colchicine and provokes an appropriate orientation for a hydrogen bond with Lys352 of β-tubulin domain. The *N*-methylated group fits in a sub-pocket with a hydrophobic contact with Leu248 whereas docking of compounds **11d** (Fig. 6c) tolerates many binding poses but decreasing the shape complementarity and without gaining by hydrogen bonding from the amide group. These results suggested that 3-hydroxy-4-methoxyphenyl core and *N*-methyl amide substitution might be crucial for binding interactions and provided a possible explanation why compound **12d** displayed potent tubulin inhibitory activity with regard to its counterpart **11d**.

3. Conclusion

We have designed and synthesized 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones which can be considered as conformationally restricted CA-4 analogues. The 3-hydroxy-4-methoxyphenyl derivatives **11d** and **12d** were potent *in vitro* tubulin polymerization inhibitors but only the *N*-methyl substituted counterpart **12d** displayed strong growth inhibitions toward the NCI-60 human cancer cell lines panel. The best antitubulin activity of compound **12d** could be explained by modeling studies which have shown that the *N*-methylated amide group fits in a sub-pocket with a hydrophobic contact with Leu248 which is not recovered with the unsubstituted analogue **11d**. However, the total loss of cellular activity of compound **11d** might be explained by bad physicochemical properties as solubility, membrane permeability or log *P*. Interestingly, the 3-amino-4-methoxy derivative **12g** displayed a growth inhibition potential as strong as compound **12d** but without inhibition of tubulin assembly at concentrations as high as 10 μM. Further experiments will be necessary to better investigate the mechanism of action of such compound. Compound **12d** has shown remarkable activity in the HUVEC shape change assays as cell invasion and endothelial tube formation experiments which are good indicators for potential *in vivo* antivascular activity. These results suggest that compound **12d** might be lead compound for the development of novel vascular disrupting agents, and is promising candidates for *in vivo* evaluation.

4. Experimental protocols

4.1. Chemistry and chemical methods

General methods: All reagents and solvents were purchased and used without further purification. Melting points were determined on a BÜCHI B-540 apparatus and are uncorrected. NMR

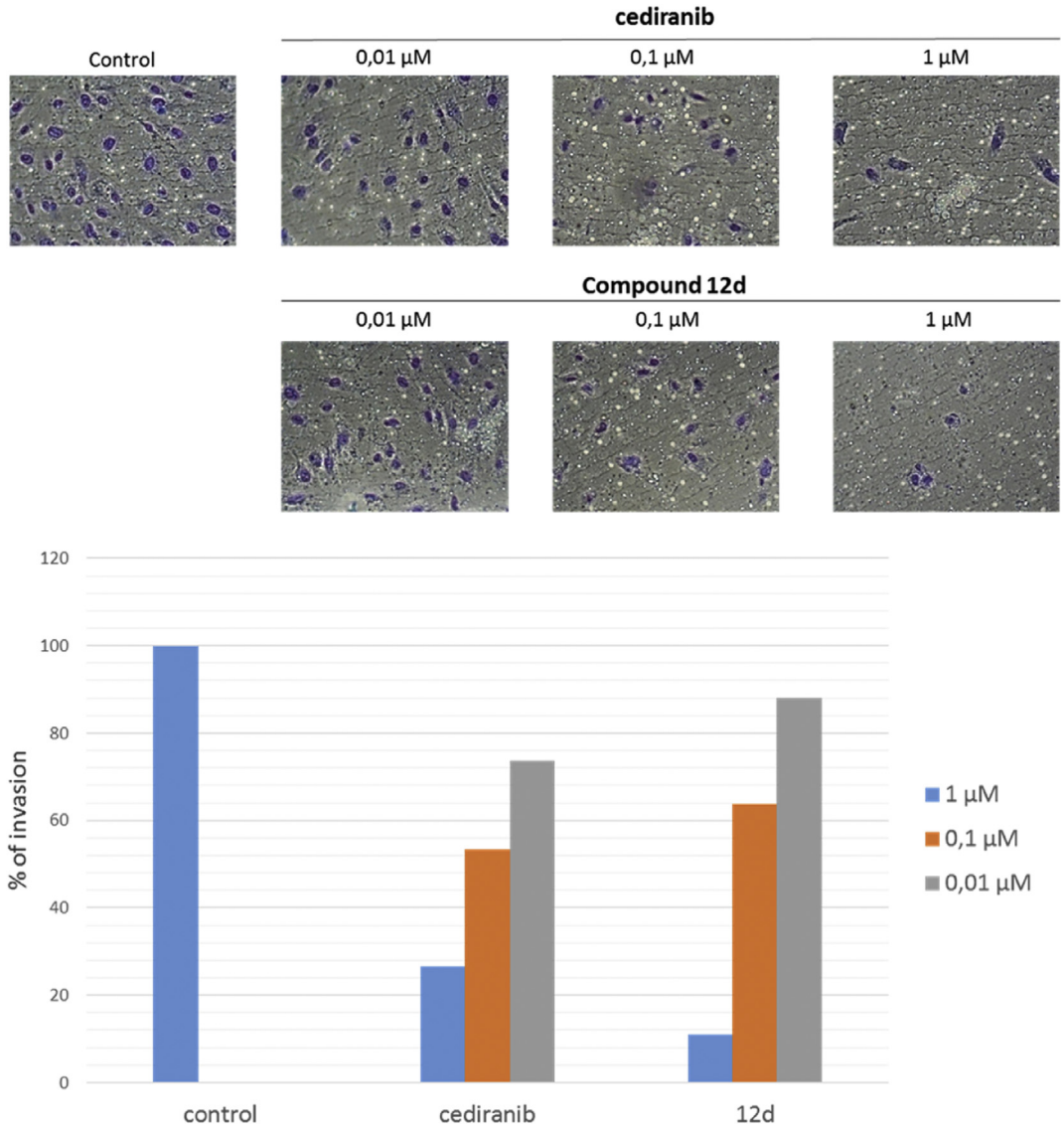


Fig. 3. Invasion inhibition on HUVEC in a Boyden chamber assay of cediranib and compound **12d**. Images depicting the penetration of HUVEC through the filter membrane. The number of HUVEC that penetrated the membrane was quantified.

spectra were recorded on a Bruker Avance 300 spectrometer operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). Chemical shifts are in parts per million (ppm) and were referenced to the residual proton peaks in deuterated solvents. Mass spectra were recorded with an LCMS (Waters Alliance Micromass ZQ 2000). LCMS analysis was performed using a Waters XBridge C18 column (5 µm particle size column, dimensions 50 mm × 4.6 mm). A gradient starting from 98% H_2O/formate buffer 5 mM (pH 3.8) and reaching 100% CH_3CN/formate buffer 5 mM (pH 3.8) within 4 min at a flow rate of 2 mL/min was used followed by a return to the starting conditions within 1 min. Elemental analyses were performed by UMR CNRS8181, and were in agreement with the calculated values within ±0.2%.

*4.1.1. Methyl 2-isothiocyanatobenzoate (**2**)*

A solution of methyl anthranilate (**1**) (15 g, 100 mmol) in dichloromethane (100 mL) was added dropwise to a mixture of thiophosgene (13.8 g, 120 mmol) in water/dichloromethane (70/150 mL) and the reaction mixture was stirred at room temperature for 12 h. The resulting solution was diluted with water (200 mL), extracted with dichloromethane (3 × 100 mL) and the organic layers were washed with water (2 × 200 mL), dried (MgSO4), filtered and concentrated *in vacuo* giving a light yellow oil, in 94% yield, which was used in the next reaction without purification.

*4.1.2. General procedure for synthesis of 2-mercaptoquinazolin-4-ones (**3**) and (**4**)*

A solution of benzylamine (1.17 g, 11 mmol) or 40% aqueous methylamine (0.85 mL, 10 mmol) in toluene (15 mL) was added dropwise to a solution of isothiocyanate **2** (10 mmol) in toluene (15 mL) at room temperature and the reaction mixture was refluxed for 12 h. After cooling, the resulting precipitate was filtered, washed with diethyl ether and dried.

*4.1.2.1. 3-Benzyl-2-mercapto-quinazolin-4-one (**3**).* White solid; 71% yield; $C_{15}H_{12}N_2OS$; MW = 268.3 g/mol; m.p. 248–250 °C (Lit. 248 °C [44]); ^{1}H NMR (300 MHz, DMSO) δ 5.66 (s, 2H, CH_2), 7.19–7.37 (m, 7H, C*H* benz, C*H* quina), 7.77 (td, 1H, J = 1.8 Hz, 8.6 Hz, C*H* quina), 7.96 (dd, 1H, J = 1.8 Hz, 8.15 Hz, C*H* quina), 12.98 (s, 1H, S*H*). LC/MS (ESI+): *m/z* = 269.3 [M+H]+.

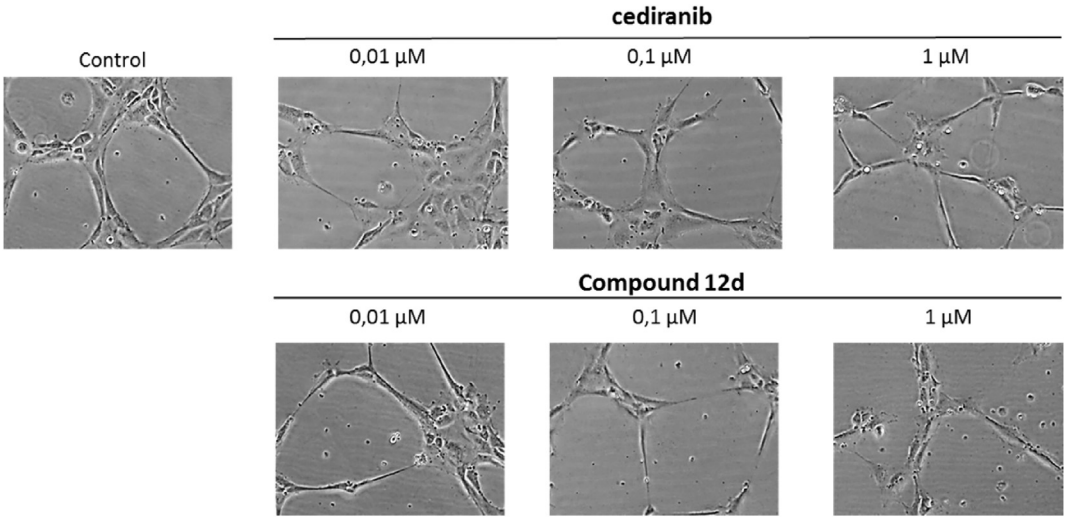


Fig. 4. Effect of cediranib and compound **12d** on tube formation. Images depicting the formation of HUVEC capillary-like tubular network by treatment with cediranib or compound **12d**.

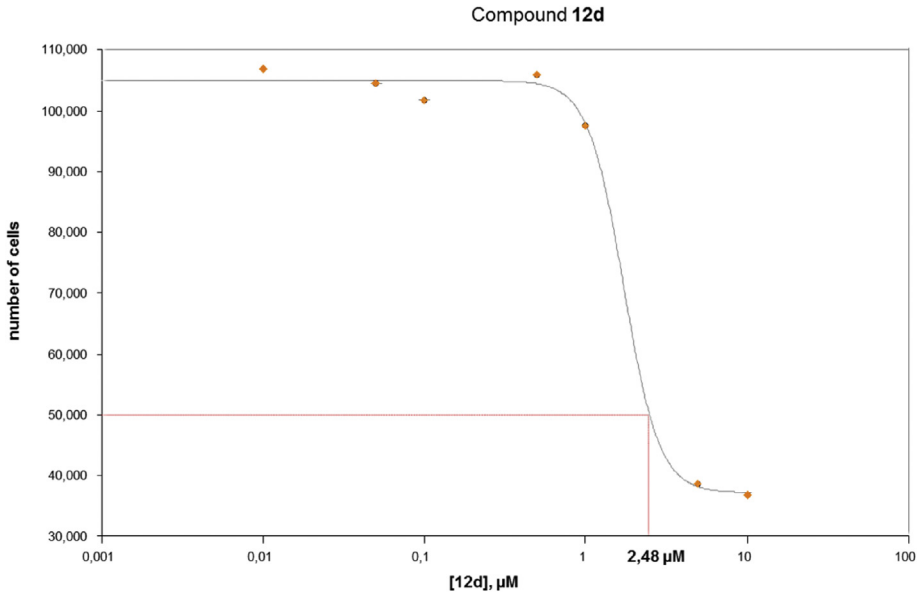


Fig. 5. Antiproliferative activity (IC_{50} µM) of compounds **12d** on HUVEC line by MTS assay.

*4.1.2.2. 2-Mercapto-3-methyl-quinazolin-4-one (**4**).* White solid; 85% yield; $C_9H_8N_2OS$; MW = 192.2 g/mol; m.p. 230–232 °C (Lit. 260–261 °C [45]); ^{1}H NMR (300 MHz, DMSO) δ 3.65 (s, 3H, C*H*$_3$), 7.29–7.40 (m, 2H, C*H* quina), 7.72 (t, 1H, J = 8.15 Hz, C*H* quina), 7.95 (d, 1H, J = 8.15 Hz, C*H* quina), 12.89 (s, 1H, *SH*). LC/MS (ESI+): *m/z* = 193.2 [M+H]+.

*4.1.3. General procedure for synthesis of 2-hydrazinoquinazolin-4-ones (**5**) and (**6**)*

A mixture of thiol **3** (0.54 g, 2 mmol) or **4** (0.38 g, 2 mmol) and hydrazine hydrate (0.93 mL, 30 mmol) in ethanol (20 mL) was refluxed for 20 h. After cooling, the resulting precipitate was filtered, washed with diethyl ether and dried.

*4.1.3.1. 3-Benzyl-2-hydrazino-quinazolin-4-one (**5**).* White solid; 86% yield; $C_{15}H_{14}N_4O$; MW = 266.3 g/mol; m.p. 244–246 °C (Lit. 242–245 °C [46]); ^{1}H NMR (300 MHz, DMSO) δ 4.66 (s, 2H, N*H*$_2$), 5.24 (s, 2H, C*H*$_2$), 7.12 (t, 1H, J = 7.80 Hz, C*H* quina), 7.17–7.38 (m, 6H, C*H* benz, C*H* quina), 7.62 (td, 1H, J = 1.60 Hz, 7.80 Hz, C*H* quina), 7.94 (dd, 1H, J = 1.60 Hz, 8.10 Hz, C*H* quina), 8.23 (s, 1H, N*H*); LC/MS (ESI+): *m/z* = 267.3 [M+H]+.

*4.1.3.2. 2-Hydrazino-2-methyl-quinazolin-4-one (**6**).* Light yellow solid; 89% yield; $C_9H_{10}N_4O$; MW = 190.2 g/mol; m.p. 212–214 °C (Lit. 209–210 °C [45]); ^{1}H NMR (300 MHz, DMSO) δ 3.33 (s, 3H, C*H*$_3$), 4.56 (s, 2H, N*H*$_2$), 7.12 (t, 1H, J = 7.40 Hz, C*H* quina), 7.31 (d, 1H, J = 7.95 Hz, C*H* quina), 7.59 (t, 1H, J = 7.40 Hz), 7.91 (dd, 1H, J = 1.30 Hz, 7.90 Hz), 8.26 (s, 1H); LC/MS (ESI+): *m/z* = 191.2 [M+H]+.

*4.1.4. General procedure for synthesis of hydrazinones (**7a-g**, **8a-g**)*

A mixture of hydrazine **5** (0.5 g, 1.88 mmol) or **6** (0.35 g, 1.88 mmol), the corresponding benzaldehyde (1.88 mmol) and 3 drops of acetic acid was refluxed overnight in methanol. After cooling, the reaction mixture was filtered and the resulting precipitate was washed with methanol and dried.

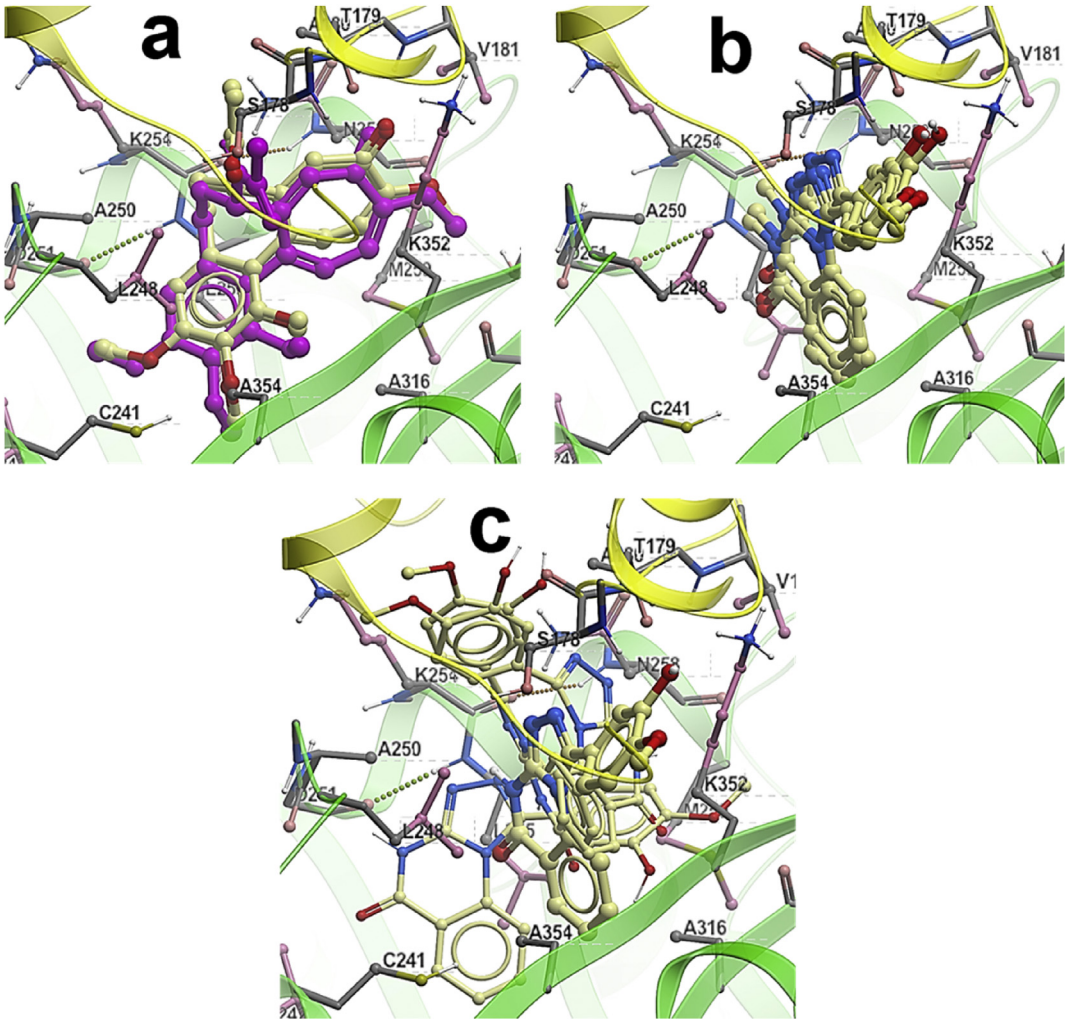


Fig. 6. Docking studies into the colchicine binding site of tubulin of (a) colchicine, (b) compound **12d** and (c) compound **11d**.

*4.1.4.1. 3-Benzyl-2-[N′-(4-methoxybenzylidene)-hydrazino]-quinazolin-4-one (**7a**).* Obtained by condensation with commercial available 4-anisaldehyde. Light yellow solid; 79% yield; $C_{23}H_{20}N_4O_2$; MW = 384.4 g/mol; m.p. 175–177 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.81 (s, 3H, OC*H*$_3$), 5.29 (s, 2H, C*H*$_2$), 7.00 (d, 2H, J = 8.95 Hz, Ar*H*), 7.13 (td, 1H, J = 2.15 Hz, 8.0 Hz, C*H* quina), 7.22 (t, 1H, J = 7.0 Hz, C*H* benz), 7.29 (t, 2H, J = 6.9 Hz, C*H* benz), 7.38 (d, 2H, 7.2 Hz, C*H* benz), 7.63 (m, 2H, C*H* quina), 7.92 (m, 3H, Ar*H*, C*H* quina), 8.31 (s, 1H, N=C*H*), 10.51 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 43.9, 55.7, 113.9, 114.4, 116.0, 122.3, 127.3, 127.7, 128.0, 128.2, 128.7, 130.0, 135.4, 137.9, 140.1, 149.8, 153.6, 160.8, 161.1. $t_{R,LCMS}$ = 3.15 min (100%); MS (ESI+): *m*/*z* = 385 [M+H]+.

*4.1.4.2. 3-Benzyl-2-[N′-(3,4-dimethoxybenzylidene)-hydrazino]-quinazolin-4-one (**7b**).* Obtained by condensation with commercial available veratraldehyde. White solid; 84% yield; $C_{24}H_{22}N_4O_3$; MW = 414.4 g/mol; m.p. 171–173 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.80 (s, 3H, OC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$), 5.30 (s, 2H, C*H*$_2$), 7.00 (d, 1H, J = 8.3 Hz, Ar*H*), 7.14 (td, 1H, J = 1.3 Hz, 7.9 Hz, C*H* quina), 7.22 (t, 1H, J = 7.2 Hz, C*H* benz), 7.30 (t, 2H, J = 7.0 Hz, C*H* benz), 7.36–7.40 (m, 3H, C*H* benz, Ar*H*), 7.57–7.64 (m, 2H, C*H* quina), 7.68 (d, 1H, J = 1.8 Hz, Ar*H*), 7.91 (dd, 1H, J = 1.25 Hz, 8.1 Hz, C*H* quina), 8.29 (s, 1H, N=C*H*), 10.56 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 43.9, 55.9, 56.0, 110.5, 111.7, 113.9, 116.0, 122.3, 122.7, 127.3, 127.7, 128.0, 128.4, 128.7, 135.4, 137.9, 140.0, 149.3, 149.8, 151.0, 153.8, 160.8. $t_{R,LCMS}$ = 3.12 min (100%); MS (ESI+): *m*/*z* = 415 [M+H]+.

*4.1.4.3. 3-Benzyl-2-[N′-(3,4,5-trimethoxybenzylidene)-hydrazino]-quinazolin-4-one (**7c**).* Obtained by condensation with commercial available 3,4,5-trimethoxybenzaldehyde. White solid; 86% yield; $C_{25}H_{24}N_4O_4$; MW = 444.5 g/mol; m.p. 209–211 °C; 1H NMR (300 MHz, DMSO) δ 3.70 (s, 3H, OC*H*$_3$), 3.86 (s, 6H, OC*H*$_3$), 5.30 (s, 2H, C*H*$_2$), 7.15 (t, 1H, J = 7.95 Hz, C*H* quina), 7.22 (t, 1H, J = 7.0 Hz, C*H* benz), 7.27–7.32 (m, 4H, C*H* benz, Ar*H*), 7.39 (d, 2H, J = 8.5 Hz, C*H* benz), 7.56–7.67 (m, 2H, C*H* quina), 7.93 (dd, 1H, J = 1.3 Hz, 7.95 Hz, C*H* quina), 8.30 (s, 1H, N=C*H*), 10.61 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 44.0, 56.4, 60.5, 105.9, 114.0, 116.1, 122.4, 127.4, 127.8, 128.0, 128.7, 131.0, 135.4, 137.8, 139.5, 139.9, 150.1, 153.5, 153.7, 160.8. $t_{R,LCMS}$ = 3.01 min (100%); MS (ESI+): *m*/*z* = 445 [M+H]+.

*4.1.4.4. 3-Benzyl-2-[N′-(3-benzyloxy-4-methoxybenzylidene)-hydrazino]-quinazolin-4-one (**7d**).* Obtained by condensation with synthesized 3-benzyloxy-4-methoxybenzaldehyde [47]. White solid; 81% yield; $C_{30}H_{26}N_4O_3$; MW = 490.5 g/mol; m.p. 185–187 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.81 (s, 3H, OC*H*$_3$), 5.18 (s, 2H, C*H*$_2$), 5.29 (s, 2H, C*H*$_2$), 7.03 (d, 1H, J = 8.3 Hz, Ar*H*), 7.14 (t, 1H, J = 7.9 Hz, C*H* quina), 7.22–7.43 (m, 9H, C*H* benz, Ar*H*), 7.51 (d, 2H, J = 8.1 Hz, C*H* benz), 7.62 (m, 2H, C*H* quina), 7.80 (d, 1H, J = 1.5 Hz, Ar*H*), 7.92 (d, 1H, J = 7.8 Hz, C*H* quina), 8.28 (s, 1H, N=C*H*), 10.54 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 43.9, 56.0, 70.4, 112.0, 112.2, 114.0, 116.1, 122.3, 123.0, 127.4, 127.8, 128.0, 128.3, 128.4, 128.5, 128.7, 128.9, 135.4, 137.4, 137.8, 140.0, 148.3, 149.8, 151.2, 153.7, 160.8. $t_{R,LCMS}$ = 3.23 min (10%), 3.37 min (90%); MS (ESI+): *m*/*z* = 491

[M+H]+.

*4.1.4.5. 3-Benzyl-2-[N′-(4-benzyloxy-3-methoxybenzylidene)-hydrazino]-quinazolin-4-one (**7e**).* Obtained as a mixture of geometric isomers by condensation with synthesized 4-benzyloxy-3-methoxybenzaldehyde [47]. White solid; 93% yield; $C_{30}H_{26}N_4O_3$; MW = 490.5 g/mol; m.p. 184–186 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.87 (s, 3H, OC*H*$_3$), 5.14 (s, 2H, C*H*$_2$), 5.29 (s, 2H, C*H*$_2$), 7.09 (d, 1H, J = 8.5 Hz, Ar*H*), 7.14 (t, 1H, J = 7.9 Hz, C*H* quina), 7.22–7.42 (m, 9H, C*H* benz, Ar*H*), 7.46 (d, 2H, J = 8.1 Hz, C*H* benz), 7.60 (m, 2H, C*H* quina), 7.69 (d, 1H, J = 1.8 Hz, Ar*H*), 7.92 (dd, 1H, J = 1.3 Hz, 8.0 Hz, C*H* quina), 8.29 (s, 1H, N=C*H*), 10.56 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 43.9, 56.1, 70.3, 111.0, 113.4, 114.0, 116.1, 122.3, 122.5, 127.4, 127.7, 128.0, 128.4, 128.7, 128.9, 135.4, 137.3, 137.8, 140.0, 149.6, 149.8, 149.9, 153.7, 160.8. $t_{R,LCMS}$ = 3.23 min (10%), 3.36 min (90%); MS (ESI+): *m*/*z* = 491 [M+H]+.

*4.1.4.6. 3-Benzyl-2-[N′-(3,4-dibenzyloxybenzylidene)-hydrazino]-quinazolin-4-one (**7f**).* Obtained as a mixture of geometric isomers by condensation with synthesized 3,4-dibenzyloxybenzaldehyde [48]. White solid; 91% yield; $C_{36}H_{30}N_4O_3$; MW = 566.6 g/mol; m.p. 146–148 °C; ^{1}H NMR (300 MHz, DMSO) δ 5.19 (s, 2H, C*H*$_2$), 5.23 (s, 2H, C*H*$_2$), 5.29 (s, 2H, C*H*$_2$), 7.11–7.17 (m, 2H, Ar*H*, C*H* quina), 7.22–7.51 (m, 16H, C*H* benz, Ar*H*), 7.58–7.68 (m, 2H, C*H* quina), 7.81 (d, 1H, J = 1.8 Hz, Ar*H*), 7.92 (dd, 1H, J = 1.2 Hz, 7.95 Hz, C*H* quina), 8.27 (s, 1H, N=C*H*), 10.53 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 42.6, 69.0, 69.2, 111.9, 112.6, 112.8, 114.7, 120.9, 121.5, 126.0, 126.4, 126.6, 126.7, 126.8, 126.9, 126.95, 127.3, 127.5, 134.1, 136.1, 136.2, 136.5, 138.6, 147.3, 148.5, 149.0, 152.1, 159.4. $t_{R,LCMS}$ = 3.52 min (10%), 3.60 min (90%); MS (ESI+): *m*/*z* = 567 [M+H]+.

*4.1.4.7. 3-Benzyl-2-[N′-(4-methoxy-3-nitrobenzylidene)-hydrazino]-quinazolin-4-one (**7g**).* Obtained as a mixture of geometric isomers by condensation with synthesized 4-methoxy-3-nitrobenzaldehyde [49]. Yellow solid; 93% yield; $C_{23}H_{19}N_5O_4$; MW = 429.4 g/mol; m.p. 237–239 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.97 (s, 3H, OC*H*$_3$), 5.30 (s, 2H, C*H*$_2$), 7.16–7.32 (m, 4H, C*H* quina, C*H* benz), 7.37–7.45 (m, 3H, C*H* benz, Ar*H*), 7.59–7.67 (m, 2H, C*H* quina), 7.92 (d, 1H, J = 7.5 Hz, C*H* quina), 8.18 (d, 1H, J = 8.1 Hz, Ar*H*), 8.38 (s, 1H, N=C*H*), 8.51 (d, 1H, J = 1.25 Hz, Ar*H*), 10.64 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 44.0, 57.4, 114.0, 114.7, 116.1, 122.5, 123.5, 126.8, 127.4, 127.8, 128.0, 128.5, 128.7, 128.8, 134.0, 135.5, 137.7, 139.9, 140.3, 150.6, 151.4, 152.8, 160.7. $t_{R,LCMS}$ = 2.89 min (15%), 3.07 min (85%); MS (ESI+): *m*/*z* = 430 [M+H]+.

*4.1.4.8. 2-[N′-(4-Methoxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8a**).* Obtained by condensation with commercial available 4-anisaldehyde. White solid; 86% yield; $C_{17}H_{16}N_4O_2$; MW = 308.3 g/mol; m.p. 187–189 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.37 (s, 3H, NC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$), 7.01 (d, 2H, J = 8.7 Hz, Ar*H*), 7.12 (td, 1H, J = 2.05 Hz, 8.0 Hz, C*H* quina), 7.61 (m, 2H, C*H* quina), 7.91 (m, 3H, C*H* quina, Ar*H*), 8.36 (s, 1H, N=C*H*), 10.46 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 55.7, 113.9, 114.4, 115.9, 122.1, 127.6, 128.4, 129.9, 135.1, 139.9, 150.2, 153.4, 160.9, 161.1. $t_{R,LCMS}$ = 2.60 min (100%); MS (ESI+): *m*/*z* = 309 [M+H]+.

*4.1.4.9. 2-[N′-(3,4-Dimethoxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8b**).* Obtained by condensation with commercial available veratraldehyde. White solid; 79% yield; $C_{18}H_{18}N_4O_3$; MW = 338.3 g/mol; m.p. 208–210 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.38 (s, 3H, NC*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 7.01 (d, 1H, J = 8.0 Hz, Ar*H*), 7.13 (td, 1H, J = 1.4 Hz, 7.95 Hz, C*H* quina), 7.37 (dd, 1H, J = 1.85 Hz, 8.35 Hz, Ar*H*), 7.55–7.65 (m, 2H, C*H* quina), 7.71 (d, 1H, J = 1.8 Hz, Ar*H*), 7.91 (dd, 1H, J = 1.3 Hz, 7.95 Hz, C*H* quina), 8.34 (s, 1H, N=C*H*), 10.52 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 56.0, 56.1, 110.4, 111.7, 114.0, 115.9, 122.1, 122.7, 127.6, 128.5, 135.1, 139.8, 149.3, 150.2, 151.0, 153.6, 160.9. $t_{R,LCMS}$ = 2.41 min (100%); MS (ESI+): *m*/*z* = 339 [M+H]+.

*4.1.4.10. 2-[N′-(3,4,5-Trimethoxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8c**).* Obtained by condensation with commercial available 3,4,5-trimethoxybenzaldehyde. White solid; 83% yield; $C_{19}H_{20}N_4O_4$; MW = 368.4 g/mol; m.p. 196–198 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.39 (s, 3H, NC*H*$_3$), 3.71 (s, 3H, OC*H*$_3$), 3.87 (s, 6H, OC*H*$_3$), 7.14 (td, 1H, J = 1.2 Hz, 8.05 Hz, C*H* quina), 7.31 (s, 2H, Ar*H*), 7.54–7.62 (m, 2H, C*H* quina), 7.92 (d, 1H, J = 7.65 Hz, C*H* quina), 8.35 (s, 1H, N=C*H*), 10.58 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 56.4, 60.5, 105.8, 114.0, 116.0, 122.2, 127.6, 131.2, 135.1, 139.5, 139.7, 150.6, 153.4, 153.5, 160.9. $t_{R,LCMS}$ = 2.46 min (100%); MS (ESI+): *m*/*z* = 369 [M+H]+.

*4.1.4.11. 2-[N′-(3-Benzyloxy-4-methoxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8d**).* Obtained by condensation with synthesized 3-benzyloxy-4-methoxybenzaldehyde [47]. White solid; 88% yield; $C_{24}H_{22}N_4O_3$; MW = 414.5 g/mol; m.p. 160–162 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.39 (s, 3H, NC*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 5.20 (s, 2H, C*H*$_2$), 7.05 (d, 1H, J = 8.3 Hz, Ar*H*), 7.13 (td, 1H, J = 1.2 Hz, 7.9 Hz, C*H* quina), 7.35–7.44 (m, 4H, C*H* benz, Ar*H*), 7.52 (m, 2H, C*H* benz), 7.56–7.63 (m, 2H, C*H* quina), 7.83 (d, 1H, J = 1.6 Hz, Ar*H*), 7.92 (d, 1H, J = 7.8 Hz, C*H* quina), 8.33 (s, 1H, N=C*H*), 10.50 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 56.0, 70.5, 112.0, 112.1, 114.0, 115.9, 122.1, 123.0, 127.6, 128.4, 128.5, 128.5, 128.8, 135.1, 137.4, 139.8, 148.4, 150.2, 151.2, 153.4, 160.9. $t_{R,LCMS}$ = 2.93 min (96%); MS (ESI+): *m*/*z* = 415 [M+H]+.

*4.1.4.12. 2-[N′-(4-Benzyloxy-3-methoxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8e**).* Obtained by condensation with synthesized 4-benzyloxy-3-methoxybenzaldehyde [47]. White solid; 94% yield; $C_{24}H_{22}N_4O_3$; MW = 414.5 g/mol; m.p. 191–193 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.38 (s, 3H, NC*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 5.14 (s, 2H, C*H*$_2$), 7.09–7.15 (m, 2H, C*H* quina, Ar*H*), 7.33–7.43 (m, 4H, C*H* benz, Ar*H*), 7.47 (d, 2H, J = 6.9 Hz, C*H* benz), 7.55–7.64 (m, 2H, C*H* quina), 7.71 (d, 1H, J = 1.6 Hz, Ar*H*), 7.91 (d, 1H, J = 7.6 Hz, C*H* quina), 8.34 (s, 1H, N=C*H*), 10.52 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 56.2, 70.3, 110.9, 113.4, 114.0, 115.9, 122.1, 122.5, 127.6, 128.3, 128.8, 128.9, 135.1, 137.3, 139.8, 149.7, 149.9, 150.2, 153.5, 160.9. $t_{R,LCMS}$ = 2.95 min (97%); MS (ESI+): *m*/*z* = 415 [M+H]+.

*4.1.4.13. 2-[N′-(3,4-Dibenzyloxybenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8f**).* Obtained by condensation with synthesized 3,4-dibenzyloxybenzaldehyde [48]. White solid; 91% yield; $C_{30}H_{26}N_4O_3$; MW = 490.5 g/mol; m.p. 158–160 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.38 (s, 3H, NC*H*$_3$), 5.19 (s, 2H, C*H*$_2$), 5.24 (s, 2H, C*H*$_2$), 7.11–7.15 (m, 2H, C*H* quina, Ar*H*), 7.31–7.41 (m, 7H, C*H* benz, Ar*H*), 7.44–7.53 (m, 4H, C*H* benz), 7.56–7.64 (m, 2H, C*H* quina), 7.83 (d, 1H, J = 1.8 Hz, Ar*H*), 7.91 (dd, 1H, J = 1.3 Hz, 8.0 Hz, C*H* quina), 8.32 (s, 1H, N=C*H*), 10.49 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 70.3, 70.6, 113.1, 114.0, 114.2, 115.9, 122.2, 122.9, 127.6, 127.8, 127.9, 128.0, 128.2, 128.2, 128.3, 128.8, 128.9, 135.1, 137.5, 137.6, 139.8, 148.7, 150.3, 153.3, 160.9. $t_{R,LCMS}$ = 3.32 min (100%); MS (ESI+): *m*/*z* = 491 [M+H]+.

*4.1.4.14. 2-[N′-(4-methoxy-3-nitrobenzylidene)-hydrazino]-3-methyl-quinazolin-4-one (**8g**).* Obtained by condensation with synthesized 4-methoxy-3-nitrobenzaldehyde [49]. Yellow solid; 86% yield; $C_{17}H_{15}N_5O_4$; MW = 353.3 g/mol; m.p. 215–217 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.39 (s, 3H, NC*H*$_3$), 3.98 (s, 3H, OC*H*$_3$), 7.15 (t, 1H, J = 7.7 Hz, C*H* quina), 7.44 (d, 1H, J = 8.65 Hz, Ar*H*), 7.56–7.67 (m, 2H, C*H* quina), 7.92 (d, 1H, J = 7.35 Hz, C*H* quina), 8.19 (dd, 1H, J = 2.1 Hz, 8.7 Hz, Ar*H*), 8.42 (s, 1H, N=C*H*), 8.52 (d, 1H, J = 1.9 Hz,

Ar*H*), 10.59 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 28.3, 57.4, 114.0, 114.7, 116.0, 122.3, 123.5, 127.6, 128.6, 133.9, 135.1, 139.7, 140.3, 151.0, 151.1, 152.8, 160.9. $t_{R,LCMS}$ = 2.58 min (100%); MS (ESI+): *m*/*z* = 354 [M+H]+.

*4.1.5. General procedure for synthesis of triazoloquinazolinones (**9a-g**, **10a-g**)*

A mixture of hydrazone **7a-g** or **8a-g** (1.5 mmol) and a 2 M aqueous solution of $FeCl_3$ (1.2 mL, 2.4 mmol) was refluxed for 12 h in ethanol (80 mL). After cooling, the reaction mixture was filtered and the resulting precipitate was washed with water, dried and recrystallized from ethanol.

*4.1.5.1. 4-Benzyl-1-(4-methoxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9a**).* White solid; 90% yield; $C_{23}H_{18}N_4O_2$; MW = 382.4 g/mol; m.p. 228–230 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.87 (s, 3H, OC*H*$_3$), 5.42 (s, 2H, C*H*$_2$), 7.13 (d, 1H, J = 8.3 Hz, C*H* quina), 7.19 (d, 2H, J = 8.8 Hz, Ar*H*), 7.28–7.37 (m, 3H, C*H* benz), 7.49–7.56 (m, 3H, C*H* benz, C*H* quina), 7.61–7.68 (m, 3H, C*H* quina, Ar*H*), 8.26 (dd, 1H, J = 1.1 Hz, 7.65 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 55.8, 115.0, 115.9, 117.6, 120.9, 127.0, 128.0, 128.5, 128.8, 129.7, 131.8, 134.3, 134.9, 136.6, 148.4, 148.8, 158.6, 161.3. $t_{R,LCMS}$ = 2.50 min (100%); MS (ESI+): *m*/*z* = 383 [M+H]+.

*4.1.5.2. 4-Benzyl-1-(3,4-dimethoxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9b**).* White solid; 91% yield; $C_{24}H_{20}N_4O_3$; MW = 412.4 g/mol; m.p. 240–241 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.73 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 5.44 (s, 2H, C*H*$_2$), 7.15 (d, 1H, J = 8.35 Hz, C*H* quina), 7.22 (t, 2H, J = 8.1 Hz, C*H* benz), 7.25–7.36 (m, 4H, Ar*H*, C*H* benz), 7.48–7.55 (m, 3H, C*H* quina, C*H* benz), 7.68 (t, 1H, J = 8.65 Hz, C*H* quina), 8.26 (dd, 1H, J = 1.35 Hz, 7.95 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 56.0, 56.1, 112.4, 113.5, 116.1, 117.6, 120.8, 123.1, 127.1, 128.0, 128.5, 128.8, 129.7, 134.3, 135.0, 136.6, 148.5, 148.7, 149.3, 151.0, 158.6. $t_{R,LCMS}$ = 2.36 min (98%); MS (ESI+): *m*/*z* = 413 [M+H]+.

*4.1.5.3. 4-Benzyl-1-(3,4,5-trimethoxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9c**).* White solid; 90% yield; $C_{25}H_{22}N_4O_4$; MW = 442.5 g/mol; m.p. 270–271 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.76 (s, 6H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 5.44 (s, 2H, C*H*$_2$), 7.03 (s, 2H, Ar*H*), 7.16 (d, 1H, J = 8.25 Hz, C*H* quina), 7.28–7.37 (m, 3H, C*H* benz), 7.48–7.57 (m, 3H, C*H* quina, C*H* benz), 7.72 (td, 1H, J = 1.65 Hz, 8.85 Hz, C*H* quina), 8.27 (dd, 1H, J = 1.45 Hz, 7.95 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.2, 56.8, 60.8, 108.2, 116.3, 117.6, 123.9, 127.1, 128.0, 128.6, 128.8, 129.7, 134.2, 135.0, 136.6, 140.1, 148.3, 148.8, 153.8, 158.6. $t_{R,LCMS}$ = 2.43 min (100%); MS (ESI+): *m*/*z* = 443 [M+H]+.

*4.1.5.4. 4-Benzyl-1-(3-benzyloxy-4-methoxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9d**).* White solid; 80% yield; $C_{30}H_{24}N_4O_3$; MW = 488.5 g/mol; m.p. 189–191 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.89 (s, 3H, OC*H*$_3$), 5.06 (s, 2H, C*H*$_2$), 5.44 (s, 2H, C*H*$_2$), 7.11 (dd, 1H, J = 1.1 Hz, 8.36 Hz, C*H* quina), 7.19–7.42 (m, 11H, C*H* benz, Ar*H*), 7.48–7.55 (m, 3H, C*H* quina, C*H* benz), 7.62 (t, 1H, J = 7.05 Hz, C*H* quina), 8.26 (dd, 1H, J = 1.25 Hz, 7.8 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 56.1, 70.4, 112.7, 115.1, 116.0, 117.5, 120.8, 123.5, 127.0, 128.0, 128.3, 128.4, 128.5, 128.8, 129.7, 134.3, 135.1, 136.6, 137.1, 148.3, 148.4, 148.7, 151.4, 158.6. $t_{R,LCMS}$ = 2.81 min (100%); MS (ESI+): *m*/*z* = 489 [M+H]+.

*4.1.5.5. 4-Benzyl-1-(4-benzyloxy-3-methoxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9e**).* White solid; 80% yield; $C_{30}H_{24}N_4O_3$; MW = 488.5 g/mol; m.p. 210–212 °C; 1H NMR (300 MHz, DMSO) δ 3.74 (s, 3H, OC*H*$_3$), 5.21 (s, 2H, C*H*$_2$), 5.44 (s, 2H, C*H*$_2$), 7.15 (d, 1H, J = 8.1 Hz, C*H* quina), 7.21 (dd, 1H, J = 1.75 Hz, 8.3 Hz, Ar*H*), 7.27–7.45 (m, 8H, C*H* benz, Ar*H*), 7.49–7.56 (m, 5H, C*H* quina, C*H* benz), 7.67 (t, 1H, J = 1.55 Hz, 8.55 Hz, C*H* quina), 8.27 (dd, 1H, J = 1.35 Hz, 7.9 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 56.2, 70.4, 113.8, 113.9, 116.1, 117.6, 121.2, 123.0, 127.1, 128.0, 128.4, 128.5, 128.8, 128.9, 129.7, 134.3, 135.0, 136.6, 137.1, 148.4, 148.7, 149.6, 150.0, 158.6. $t_{R,LCMS}$ = 2.84 min (100%); MS (ESI+): *m*/*z* = 489 [M+H]+.

*4.1.5.6. 4-Benzyl-1-(3,4-dibenzyloxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9f**).* White solid; 72% yield; $C_{36}H_{28}N_4O_3$; MW = 564.6 g/mol; m.p. 225–227 °C; ^{1}H NMR (300 MHz, DMSO) δ 5.12 (s, 2H, C*H*$_2$), 5.26 (s, 2H, C*H*$_2$), 5.43 (s, 2H, C*H*$_2$), 7.05 (dd, 1H, J = 0.95 Hz, 8.3 Hz, C*H* quina), 7.22 (dd, 1H, J = 1.9 Hz, 8.25 Hz, Ar*H*), 7.27–7.44 (m, 13H, C*H* benz, Ar*H*), 7.48–7.55 (m, 4H, C*H* quina, C*H* benz), 7.59 (td, 1H, J = 1.7 Hz, 7.4 Hz, C*H* quina), 8.27 (Dd, 1H, J = 1.5 Hz, 7.6 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 70.4, 70.5, 114.7, 116.0, 117.5, 121.3, 123.6, 127.0, 127.9, 128.0, 128.1, 128.3, 128.4, 128.6, 128.8, 128.9, 129.6, 134.2, 135.0, 136.6, 137.2, 137.3, 148.3, 148.6, 148.7, 150.5, 158.6. $t_{R,LCMS}$ = 3.17 min (97%); MS (ESI+): *m*/*z* = 565 [M+H]+.

*4.1.5.7. 4-Benzyl-1-(4-methoxy-3-nitrophenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**9g**).* White solid; 66% yield; $C_{23}H_{17}N_5O_4$; MW = 427.4 g/mol; m.p. 247–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 4.05 (s, 3H, OC*H*$_3$), 5.45 (s, 2H, C*H*$_2$), 7.16 (d, 1H, J = 8.3 Hz, C*H* quina), 7.28–7.37 (m, 3H, C*H* benz), 7.49 (d, 2H, J = 6.65 Hz, C*H* benz), 7.56 (t, 1H, J = 7.4 Hz, C*H* quina), 7.63 (d, 1H, J = 8.85 Hz, Ar*H*), 7.70 (t, 1H, J = 8.65 Hz, C*H* quina), 8.01 (dd, 1H, J = 2.1 Hz, 8.7 Hz, Ar*H*), 8.28 (m, 2H, C*H* quina, Ar*H*). $t_{R,LCMS}$ = 2.46 min (98%); MS (ESI+): *m*/*z* = 428 [M+H]+.

*4.1.5.8. 1-(4-Methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10a**).* White solid; 62% yield; $C_{17}H_{14}N_4O_2$; MW = 306.3 g/mol; m.p. 216–218 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.63 (s, 3H, NC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 7.10 (d, 1H, J = 8.2 Hz, C*H* quina), 7.18 (d, 2H, J = 8.7 Hz, Ar*H*), 7.50 (t, 1H, J = 7.85 Hz, C*H* quina), 7.58–7.66 (m, 3H, C*H* quina, C*H* benz), 8.22 (dd, 1H, J = 1.3 Hz, 7.8 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 55.9, 115.1, 115.8, 117.7, 120.9, 126.9, 129.5, 131.7, 134.1, 134.7, 148.2, 149.2, 158.8, 161.3. $t_{R,LCMS}$ = 2.28 min (100%); MS (ESI+): *m*/*z* = 307 [M+H]+. Anal. Calcd (%) for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29; found C, 66.74; H, 4.58; N, 18.21.

*4.1.5.9. 1-(3,4-Dimethoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10b**).* White solid; 50% yield; $C_{18}H_{16}N_4O_3$; MW = 336.3 g/mol; m.p. 234–236 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.63 (s, 3H, NC*H*$_3$), 3.75 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 7.13 (d, 1H, J = 8.2 Hz, C*H* quina), 7.20 (m, 2H, Ar*H*)), 7.24 (s, 1H, Ar*H*), 7.50 (td, 1H, J = 0.9 Hz, 8.1 Hz, C*H* quina), 7.66 (td, 1H, J = 1.6 Hz, 8.75 Hz, C*H* quina), 8.22 (dd, 1H, J = 1.5 Hz, 7.85 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 56.1, 56.2, 112.4, 113.4, 116.0, 117.6, 120.9, 123.1, 127.0, 129.5, 134.1, 134.7, 148.3, 149.1, 149.4, 151.0, 158.8. $t_{R,LCMS}$ = 2.13 min (97%); MS (ESI+): *m*/*z* = 337 [M+H]+. Anal. Calcd (%) for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66; found C, 64.33; H, 4.81; N, 16.58.

*4.1.5.10. 1-(3,4,5-Trimethoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10c**).* White solid; 20% yield; $C_{19}H_{18}N_4O_4$; MW = 366.3 g/mol; m.p. 263–265 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.64 (s, 3H, NC*H*$_3$), 3.78 (s, 6H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 7.00 (s, 2H, Ar*H*), 7.16 (d, 1H, J = 8.3 Hz, C*H* quina), 7.52 (td, 1H, J = 0.75 Hz, 8.05 Hz, C*H* quina), 7.70 (td, 1H, J = 1.6 Hz, 8.75 Hz, C*H* quina), 8.23 (dd, 1H, J = 1.4 Hz, 7.85 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.66, 56.7, 60.8, 107.8, 116.2, 117.6, 124.0, 127.1, 129.5, 134.0, 134.9, 139.7, 148.2, 149.1, 153.8, 158.7. $t_{R,LCMS}$ = 2.22 min (100%); MS

(ESI+): m/z = 367 [M+H]+. Anal. Calcd (%) for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29; found C, 62.38; H, 5.02; N, 15.14.

*4.1.5.11. 1-(3-Benzyloxy-4-methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10d**).* White solid; 81% yield; $C_{24}H_{20}N_4O_3$; MW = 412.4 g/mol; m.p. 215–216 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.64 (s, 3H, NC*H*$_3$), 3.91 (s, 3H, OC*H*$_3$), 5.09 (s, 2H, C*H*$_2$), 7.09 (dd, 1H, J = 0.65 Hz, 8.4 Hz, C*H* quina), 7.23 (m, 2H, Ar*H*), 7.31–7.44 (m, 6H, C*H* benz, Ar*H*), 7.51 (td, 1H, J = 1.1 Hz, 8.75 Hz, C*H* quina), 7.61 (td, 1H, J = 1.7 Hz, 8.45 Hz, C*H* quina), 8.23 (dd, 1H, J = 1.35 Hz, 7.8 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 56.1, 70.4, 112.7, 115.0, 115.9, 117.6, 120.8, 123.5, 126.9, 128.2, 128.4, 128.8, 129.4, 134.0, 134.8, 137.1, 148.2, 148.3, 149.1, 151.4, 158.8. $t_{R,LCMS}$ = 2.61 min (98%); MS (ESI+): m/z = 413 [M+H]+.

*4.1.5.12. 1-(4-Benzyloxy-3-methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10e**).* White solid; 77% yield; $C_{24}H_{20}N_4O_3$; MW = 412.4 g/mol; m.p. 215–217 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.65 (s, 3H, NC*H*$_3$), 3.76 (s, 3H, OC*H*$_3$), 5.21 (s, 2H, C*H*$_2$), 7.14 (d, 1H, J = 8.05 Hz, C*H* quina), 7.19 (dd, 1H, J = 2.0 Hz, 8.2 Hz, Ar*H*), 7.27 (m, 2H, Ar*H*), 7.34–7.46 (m, 3H, C*H* benz), 7.49–7.55 (m, 3H, C*H* quina, C*H* benz), 7.66 (td, 1H, J = 1.6 Hz, 8.85 Hz, C*H* quina), 8.24 (dd, 1H, J = 1.4 Hz, 7.9 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 56.2, 70.4, 113.7, 114.0, 116.0, 117.6, 121.2, 123.0, 127.0, 128.4, 128.9, 129.5, 134.0, 134.7, 137.1, 148.2, 149.1, 149.7, 150.0, 158.7. $t_{R,LCMS}$ = 2.71 min (100%); MS (ESI+): m/z = 413 [M+H]+.

*4.1.5.13. 1-(3,4-Dibenzyloxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10f**).* White solid; 75% yield; $C_{30}H_{24}N_4O_3$; MW = 488.5 g/mol; m.p. 145–147 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.61 (s, 3H, NC*H*$_3$), 5.12 (s, 2H, C*H*$_2$), 5.25 (s, 2H, C*H*$_2$), 7.02 (d, 1H, J = 6.6 Hz, Ar*H*), 7.19 (d, 1H, J = 7.0 Hz, C*H* quina), 7.29–7.55 (m, 14H, C*H* quina, C*H* benz, Ar*H*), 8.24 (dd, 1H, J = 1.4 Hz, 7.9 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.7, 70.5, 70.6, 114.8, 115.8, 115.9, 117.6, 121.2, 123.6, 127.0, 127.9, 128.0, 128.1, 128.3, 128.4, 128.8, 128.9, 129.5, 134.0, 134.7, 137.2, 148.1, 148.7, 149.1, 150.5, 158.7. $t_{R,LCMS}$ = 3.12 min (96%); MS (ESI+): m/z = 489 [M+H]+.

*4.1.5.14. 1-(4-Methoxy-3-nitrophenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**10g**).* Light orange solid; 60% yield; $C_{17}H_{13}N_5O_4$; MW = 351.3 g/mol; m.p. 282–284 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.66 (s, 3H, NC*H*$_3$), 3.76 (s, 3H, OC*H*$_3$), 7.16 (d, 1H, J = 8.25 Hz, C*H* quina), 7.55 (t, 1H, J = 7.7 Hz, C*H* quina), 7.64 (d, 1H, J = 8.8 Hz, Ar*H*), 7.69 (t, 1H, J = 7.7 Hz, C*H* quina), 7.99 (dd, 1H, J = 2.85 Hz, 8.8 Hz, Ar*H*), 8.26 (m, 2H, C*H* quina, Ar*H*); ^{13}C NMR (75 MHz, DMSO) δ 29.7, 57.6, 115.8, 116.0, 117.6, 120.8, 126.9, 127.2, 129.6, 133.9, 135.0, 136.2, 139.6, 146.4, 149.4, 154.0, 158.8. $t_{R,LCMS}$ = 2.28 min (100%); MS (ESI+): m/z = 352 [M+H]+.

*4.1.6. General procedure for synthesis of deprotected or reduced compounds (**11a-g**, **12d-g**)*

A mixture of triazoloquinazolinone **9a-g** or **10d-g** (0.5 mmol), ammonium formate (15 mmol) and Pd/C (0.5 g) was refluxed in ethanol until starting material was disappeared (followed by TLC). The warm solution was filtered on a pad of celite and the filtrate was evaporated. The residue was recrystallized from methanol.

*4.1.6.1. 1-(4-Methoxyphenyl)-4H-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11a**).* White solid; 85% yield; $C_{16}H_{12}N_4O_2$; MW = 292.3 g/mol; m.p. 290–292 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.89 (s, 3H, OC*H*$_3$), 7.11 (dd, 1H, J = 1.1 Hz, 8.8 Hz, C*H* quina), 7.19 (d, 2H, J = 8.85 Hz, Ar*H*), 7.50 (td, 1H, J = 1.1 Hz, 8.4 Hz, C*H* quina), 7.59–7.66 (m, 3H, C*H* quina, Ar*H*), 8.20 (dd, 1H, J = 1.7 Hz, 8.0 Hz, C*H* quina), 12.93 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 55.8, 115.0, 115.9, 118.3, 121.1, 126.8, 129.3, 131.7, 134.7, 134.9, 147.0, 148.3, 159.8, 161.3. $t_{R,LCMS}$ = 1.78 min (100%); MS (ESI+): m/z = 293 [M+H]+. Anal. Calcd (%) for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.14; N, 19.17; found C, 65.84; H, 4.05; N, 19.22.

*4.1.6.2. 1-(3,4-Dimethoxyphenyl)-4H-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11b**).* White solid; 48% yield; $C_{17}H_{14}N_4O_3$; MW = 322.3 g/mol; m.p. 279–280 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.74 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 7.13 (d, 1H, J = 8.4 Hz, C*H* quina), 7.19–7.25 (m, 3H, Ar*H*), 7.48 (t, 1H, J = 7.6 Hz, C*H* quina), 7.63 (t, 1H, J = 8.0 Hz, C*H* quina), 8.18 (d, 1H, J = 7.4 Hz, C*H* quina), 12.78 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 56.1, 56.2, 112.4, 113.5, 116.1, 118.4, 121.1, 123.1, 126.8, 129.2, 134.6, 135.0, 147.0, 148.6, 149.3, 150.9, 160.0. $t_{R,LCMS}$ = 1.67 min (100%); MS (ESI+): m/z = 323 [M+H]+. Anal. Calcd (%) for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38; found C, 63.41; H, 4.27; N, 17.25.

*4.1.6.3. 1-(3,4,5-Trimethoxyphenyl)-4H-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11c**).* White solid; 75% yield; $C_{18}H_{16}N_4O_4$; MW = 352.3 g/mol; m.p. 282–283 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.77 (s, 6H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 7.01 (s, 2H, Ar*H*), 7.17 (d, 1H, J = 8.0 Hz, C*H* quina), 7.50 (td, 1H, J = 0.9 Hz, 8.25 Hz, C*H* quina), 7.68 (td, 1H, J = 1.65 Hz, 8.8 Hz, C*H* quina), 8.19 (dd, 1H, J = 1.4 Hz, 7.85 Hz, C*H* quina), 12.92 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 56.6, 60.7, 107.8, 116.4, 118.2, 124.2, 126.9, 129.2, 134.8, 134.9, 139.6, 147.0, 148.2, 153.7, 159.7. $t_{R,LCMS}$ = 1.71 min (100%); MS (ESI+): m/z = 353 [M+H]+. Anal. Calcd (%) for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.90; found C, 61.51; H, 4.63; N, 15.84.

*4.1.6.4. 1-(3-Hydroxy-4-methoxyphenyl)-4H-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11d**).* White solid; 35% yield; $C_{16}H_{12}N_4O_3$; MW = 308.3 g/mol; m.p. 296–298 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.89 (s, 3H, OC*H*$_3$), 7.03 (m, 2H, Ar*H*), 7.13 (m, 2H, C*H* quina, Ar*H*), 7.50 (t, 1H, J = 8.1 Hz, C*H* quina), 7.65 (t, 1H, J = 8.1 Hz, C*H* quina), 8.19 (d, 1H, J = 7.4 Hz, C*H* quina), 9.51 (s, 1H, O*H*), 12.86 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 56.1, 112.7, 115.9, 116.9, 118.6, 121.4, 126.6, 129.2, 134.2, 135.0, 146.7, 147.2, 149.5, 149.9, 160.7. $t_{R,LCMS}$ = 1.49 min (100%); MS (ESI+): m/z = 309 [M+H]+. Anal. Calcd (%) for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17; found C, 62.41; H, 4.01; N, 18.06.

*4.1.6.5. 1-(4-Hydroxy-3-methoxyphenyl)-4H-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11e**).* White solid; 51% yield; $C_{16}H_{12}N_4O_3$; MW = 308.3 g/mol; m.p. 280–281 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.76 (s, 3H, OC*H*$_3$), 6.98 (d, 1H, J = 8.0 Hz, Ar*H*), 7.06 (dd, 1H, J = 2.05 Hz, 8.1 Hz, Ar*H*), 7.15 (d, 1H, J = 8.3 Hz, C*H* quina), 7.21 (d, 1H, J = 1.95 Hz, Ar*H*), 7.48 (td, 1H, J = 0.95 Hz, 8.15 Hz, C*H* quina), 7.65 (td, 1H, J = 1.75 Hz, 8.9 Hz, C*H* quina), 8.18 (dd, 1H, J = 1.5 Hz, 7.85 Hz, C*H* quina), 9.67 (s, 1H, O*H*), 12.86 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO) δ 56.3, 114.1, 116.1, 116.3, 118.3, 119.5, 123.3, 126.8, 129.3, 134.7, 135.0, 147.3, 148.1, 148.3, 149.1, 159.9. $t_{R,LCMS}$ = 1.48 min (100%); MS (ESI+): m/z = 309 [M+H]+. Anal. Calcd (%) for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17; found C, 62.42; H, 3.98; N, 18.10.

*4.1.6.6. 4-Benzyl-1-(3,4-dihydroxyphenyl)-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11f**).* Beige solid; 42% yield; $C_{22}H_{16}N_4O_3$; MW = 384.4 g/mol; m.p. 291–293 °C; ^{1}H NMR (300 MHz, DMSO) δ 5.41 (s, 2H, C*H*$_2$), 6.93 (m, 2H, Ar*H*), 7.00 (s, 1H, Ar*H*), 7.20 (d, 1H, J = 8.35 Hz, C*H* quina), 7.27–7.36 (m, 3H, C*H* benz), 7.48–7.54 (m, 3H, C*H* quina, C*H* benz), 7.68 (t, 1H, J = 7.25 Hz, C*H* quina), 8.24 (d, 1H, J = 6.75 Hz, C*H* quina), 9.51 (s, 2H, O*H*); ^{13}C NMR (75 MHz, DMSO) δ 46.1, 116.0, 116.5, 117.3, 117.6, 119.4, 121.7, 126.9, 127.9, 128.5, 128.8, 129.6, 134.3, 134.9, 136.6, 146.2, 148.1, 148.6, 148.7, 158.7. $t_{R,LCMS}$ = 2.05 min (100%); MS (ESI+): m/z = 385 [M+H]+.

*4.1.6.7. 1-(3-Amino-4-methoxyphenyl)-4-Benzyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**11g**).* White solid; 71% yield; $C_{23}H_{19}N_5O_2$; MW = 397.4 g/mol; m.p. 274–276 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.87 (s, 3H, OC*H*$_3$), 5.08 (s, 2H, N*H*$_2$), 5.42 (s, 2H, C*H*$_2$), 6.80 (dd, 1H, J = 1.8 Hz, 8.0 Hz, Ar*H*), 6.86 (d, 1H, J = 1.8 Hz, Ar*H*), 7.00 (d, 1H, J = 8.3 Hz, Ar*H*), 7.22–7.36 (m, 4H, C*H* quina, Ar*H*), 7.47–7.54 (m, 3H, C*H* quina, Ar*H*), 7.68 (td, 1H, J = 1.6 Hz, 8.6 Hz, C*H* quina), 8.25 (d, 1H, J = 7.9 Hz, C*H* quina). $t_{R,LCMS}$ = 2.13 min (100%); MS (ESI+): *m/z* = 398 [M+H]+.

*4.1.6.8. 1-(3-Hydroxy-4-methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**12d**).* White solid; 36% yield; $C_{17}H_{14}N_4O_3$; MW = 322.3 g/mol; m.p. 290–291 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.63 (s, 3H, NC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 7.01–7.06 (m, 2H, Ar*H*), 7.14–7.17 (m, 2H, C*H* quina, Ar*H*), 7.50 (t, 1H, J = 7.9 Hz, C*H* quina), 7.66 (td, 1H, J = 1.7 Hz, 8.7 Hz, C*H* quina), 8.22 (dd, 1H, J = 1.5 Hz, 7.9 Hz, C*H* quina), 9.52 (s, 1H, O*H*); ^{13}C NMR (75 MHz, DMSO) δ 29.66, 56.7, 60.8, 107.8, 116.2, 117.6, 124.0, 127.1, 129.5, 134.0, 134.9, 139.7, 148.2, 149.1, 153.8, 158.7. $t_{R,LCMS}$ = 1.63 min (100%); MS (ESI+): *m/z* = 323 [M+H]+. Anal. Calcd (%) for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38; found C, 63.47; H, 4.29; N, 17.30.

*4.1.6.9. 1-(4-Hydroxy-3-methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**12e**).* White solid; 35% yield; $C_{17}H_{14}N_4O_3$; MW = 322.3 g/mol; m.p. 315–317 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.63 (s, 3H, NC*H*$_3$), 3.76 (s, 3H, OC*H*$_3$), 6.99 (d, 1H, J = 7.95 Hz, Ar*H*), 7.06 (dd, 1H, J = 1.9 Hz, 8.1 Hz, Ar*H*), 7.15 (d, 1H, J = 8.25 Hz, C*H* quina), 7.20 (d, 1H, J = 1.8 Hz, Ar*H*), 7.50 (t, 1H, J = 7.3 Hz, C*H* quina), 7.66 (td, 1H, J = 1.5 Hz, 8.7 Hz, C*H* quina), 8.21 (dd, 1H, J = 1.4 Hz, 7.9 Hz, C*H* quina), 9.70 (s, 1H, O*H*); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 56.3, 114.0, 116.0, 116.4, 117.6, 119.3, 123.3, 126.9, 129.4, 134.1, 134.7, 148.4, 148.5, 149.0, 149.2, 158.8. $t_{R,LCMS}$ = 1.61 min (100%); MS (ESI+): *m/z* = 323 [M+H]+. Anal. Calcd (%) for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38; found C, 63.41; H, 4.42; N, 17.31.

*4.1.6.10. 1-(3,4-Dihydroxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**12f**).* White solid; 37% yield; $C_{16}H_{12}N_4O_3$; MW = 308.3 g/mol; m.p. 304–305 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.62 (s, 3H, NC*H*$_3$), 6.90–6.98 (m, 3H, Ar*H*), 7.17 (d, 1H, J = 7.3 Hz, C*H* quina), 7.49 (t, 1H, J = 7.3 Hz, C*H* quina), 7.66 (t, 1H, J = 8.5 Hz, C*H* quina), 8.21 (d, 1H, J = 7.7 Hz, C*H* quina), 9.52 (s, 2H, O*H*); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 115.9, 116.6, 117.2, 117.7, 119.4, 121.6, 126.8, 129.4, 134.1, 134.6, 146.2, 148.1, 148.5, 149.0, 158.8. $t_{R,LCMS}$ = 1.48 min (100%); MS (ESI+): *m/z* = 309 [M+H]+. Anal. Calcd (%) for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17; found C, 62.42; H, 4.01; N, 18.04.

*4.1.6.11. 1-(3-Amino-4-methoxyphenyl)-4-methyl-[1,2,4]-triazolo-[4,3-a]-quinazolin-5-one (**12g**).* White solid; 22% yield; $C_{17}H_{15}N_5O_2$; MW = 321.3 g/mol; m.p. 285–287 °C; ^{1}H NMR (300 MHz, DMSO) δ 3.62 (s, 3H, NC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 5.09 (s, 2H, N*H*$_2$), 6.77 (d, 1H, J = 8.2 Hz, Ar*H*), 6.84 (s, 1H, Ar*H*), 7.00 (d, 1H, J = 7.85 Hz, Ar*H*), 7.21 (d, 1H, J = 9.0 Hz, C*H* quina), 7.50 (t, 1H, J = 7.4 Hz, C*H* quina), 7.66 (t, 1H, J = 7.95 Hz, C*H* quina), 8.21 (d, 1H, J = 7.5 Hz, C*H* quina); ^{13}C NMR (75 MHz, DMSO) δ 29.6, 55.9, 111.0, 114.9, 116.0, 117.7, 117.9, 121.1, 126.9, 129.4, 134.1, 134.6, 138.8, 148.3, 148.9, 149.0, 158.9. $t_{R,LCMS}$ = 1.71 min (96%); MS (ESI+): *m/z* = 322 [M+H]+. Anal. Calcd (%) for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79; found C, 63.65; H, 4.66; N, 21.72.

4.2. Biological assay methods

4.2.1. In vitro microtubule polymerization assay

In-vitro tubulin polymerization assays were performed on a fluorescence spectrometer (Varioskan flash, Thermo Scientific, Courtaboeuf, France), equipped with a half area 96 wells microplate (Corning Costar). Excitation and emission wavelengths were 360 and 420 nm, respectively. All reagents were purchased from Cytoskeleton (Denver, CO, USA). For each inhibitor investigated, a series of ten concentrations (from 0.1 to 10 μM) was prepared in 100 μg purified bovine tubulin and buffer containing 20% glycerol, 1 mM GTP, 80 mM PIPES (pH 6.9), 2.0 mM MgCl2, 0.5 mM EGTA and 5 nM fluorescent reporter and kept at 0 °C. Then, the medium was incubated in the plate reader, at 37 °C, for 1 h. A mixture without inhibitor exhibited an increase in fluorescent intensity as a function of incubation time corresponding to the inclusion of the probe inside the tubulin polymer. On the contrary, compounds which strongly inhibited microtubule formation were identified by a fluorescent signal remaining negligible along the experiment. The effect of inhibitor concentration on tubulin polymerization rate was studied and the IC_{50} value was defined as the concentration of product which inhibits the rate of polymerization by a factor 50. It was determined from three independent assays using GraphPad Prism software (version 4).

4.2.2. Cell proliferation on HT29 and HUVEC cell line

HT 29 Colon cancer cells were obtained from European Collection of Cell Cultures. Human umbilical vein endothelial cells (HUVECs) were purchased from Promocell. FBS, medium, penicillin-streptomycin, and other agents used in cell culture studies were purchased from Invitrogen. Cancer cell lines were cultured, at 37 °C in a CO_2 incubator, respectively in RPMI- 1640, MEM and DMEM + Glutamax-I medium supplemented with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 mg/mL). Briefly, HT29 colon cancer cells and HUVECs were plated at a density of 3×10^3 cells/well in 96-well plates for 24 h. Then the medium was removed, and cells were treated with either DMSO as a control or at a 10 μM single dose or various concentrations of compounds. The final concentration of DMSO in the medium was <0.1% (v/v). After the cells were incubated for 72 h, cell proliferation was estimated by colorimetric MTS test.

4.2.3. Cell growth inhibition

The compounds were tested against a panel of 60 human cancer cell lines at the National Cancer Institute, Rockville, MD [30]. The cytotoxicity studies were conducted using a 48 h exposure protocol using the sulforhodamine B assay [31,32].

4.2.4. Cell invasion assay

ECGM medium + SupplementMix were purchased from Promocell. *In vitro* invasion assay was carried out using invasion chamber, coated with Matrigel® matrix, with 6.4 mm diameter PET membrane (8 micron pore size, BD Biocat). The bottom chambers were filled with ECGM medium with 20 ng/mL VEGF and the top chambers were seeded with ECGM medium (without growth factors) and HUVEC (1×10^5 cells per well). The top chamber contained vehicle or compound at various concentrations (0.01, 0.1 or 1 μM). Cells were allowed to migrate for 24 h. Non migrated cells were scraped with a cotton swab, and migrated cells were fixed with 100% methanol and stained with 0.05% crystal violet. The number of HUVEC that penetrated the membrane was quantified by manual counting and photographed with Moticam®. The percentage of migrated cells was expressed on the basis of vehicle control wells.

4.2.5. In vitro endothelial tube formation assay

Geltrex® was thawed overnight at 4 °C in an ice bath, and then 100 μL of solution was used to coat 24-well plates. The plates were then incubated at 37 °C for 30 min to ensure complete gelation of the matrix. HUVEC cells were then seeded into 96-well plates at a

cell density of 50,000 cells/well and allowed to incubate for 10 h at 37 °C. Cells were treated with different concentrations of compound (0.01, 0.1 and 1 μM). The morphological changes of the cells and tubes formed were observed under inverted microscope and photographed with Moticam®.

4.3. Molecular modeling

From the 4O2B pdb entry of tubulin co-crystallized with colchicine was removed the water molecules and were only kept one α/β tubulin dimer (chains A and B) incorporating colchicine and GTP. The structure has been submitted to a refinement of the hydrogen geometry by 1000 then 5000 steps of steepest descent and conjugated gradient energy minimization with CHARMM force field [50]. Docking was performed with GOLD 5.2 [51] using a 100% automatic setting of processed genetic operations which is sufficient for these rigid molecules. Early termination of docking was activated as the 3 top scored poses fitted within 1.5 Å.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.03.056.

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