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Synthesis and biological evaluation of 3-amino-1,2,4-triazole derivatives as potential anticancer compounds

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ABSTRACT

Two series of compounds carrying 3-amino-1,2,4-triazole scaffold were synthesized and evaluated for their anticancer activity against a panel of cancer cell lines using XTT assay. The 1,2,4-triazole synthesis was revisited for the first series of pyridyl derivatives. The biological results revealed the efficiency of the 3-amino-1,2,4-triazole core that could not be replaced and a clear beneficial effect of a 3-bromophenylamino moiety in position 3 of the triazole for both series (compounds **2.6** and **4.6**) on several cell lines tested. Moreover, our results point out an antiangiogenic activity of these compounds. Overall, the 5-aryl-3-phenylamino-1,2,4-triazole structure has promising dual anticancer activity.

1. Introduction

Cancer is the second leading cause of death globally and the most prevalent one in developed countries. In 2018, it was responsible for an estimated 9.6 million deaths and over 18 million new cases were reported. Moreover, this prevalence is expected to increase in the upcoming years [1]. Despite sustained efforts in cancer research and indisputable results for some types of cancers [1,2], the main goal of durable cure remains unachieved for the majority of cancer types. One of the challenges on the way to durable anticancer cure is drug resistance phenomena exhibited by cancer cells to escape drug pressure, which leads in many cases to transient responses to the treatments [3,4]. One strategy to prevent or attenuate the appearance of strong resistance mechanisms, relies on distributing the drug pressure rather than concentrating it on a single target [5–7]. In line with this, it is appealing to utilize dual-target therapy, which consists of creating drugs blocking simultaneously two or more essential mechanisms.

Two main cancer hallmarks are proliferation and angiogenesis [8]. Proliferation is characterized by the ability of cancer cells to sustain uncontrolled replication that leads to tumor growth. While, angiogenesis is the process of new blood vessels formation developed by cancer cells, to sustain high metabolism, hypoxia and fast replication. The newly formed neovasculature facilitates the intake of increased amounts of nutrients and oxygen by the tumor and the evacuation of metabolic wastes and carbon dioxide. Therefore, drugs targeting two essential mechanisms like tumor proliferation and angiogenesis would have a real potential to display enhanced and lasting anticancer efficiency.

Over the last decades, increased research has been devoted to triazole drugs [9–11]. Among them, 3-amino-1,2,4-triazoles derivatives have attracted special attention as they demonstrated a broad spectrum of bioactivities, including potential applications against thrombotic disorders [12], fibrotic [13], and auto-immune diseases [13], central nervous system disorders [14], obesity [15], diabetes [15,16], Alzheimer's disease [16], microbial infections [14,17–19] or cancer [13,16,20–24]. Substitution of 3-amino-1,2,4-triazole (Fig. 1a) by aryl groups leads to particularly promising anticancer scaffolds. For example, the 3-pyridotriazole core I is found in several antiangiogenic compounds [20,21,23,24], as well as the phenylamino triazoles derivatives II [13,16,23,24]. A representative example of aryl-substituted 3-

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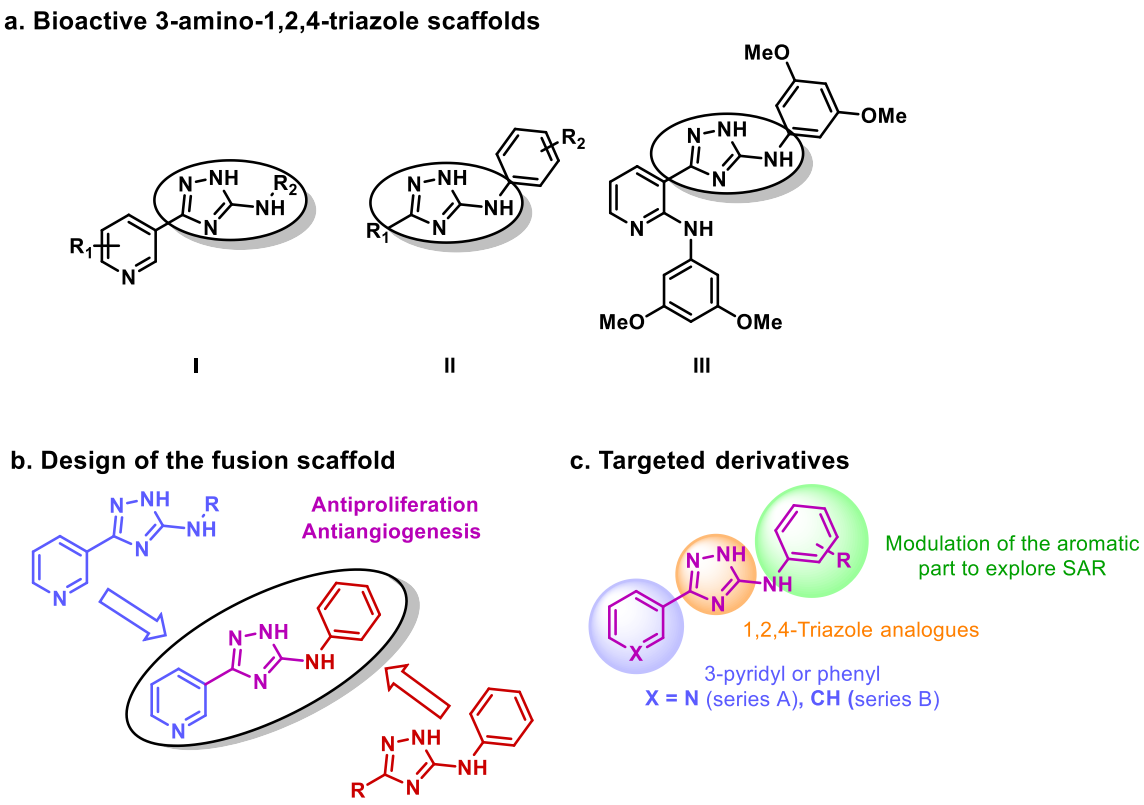


Fig. 1. (a) Structure of cores I, II, and anticancer compound III; (b) Design of the arylamino-3-pyrido-1,2,4-triazole fusion scaffold **c.** Structure of the targeted analogs.

amino-1,2,4-triazole is compound III (Fig. 1a) that shows potent antiproliferative and anti-tubulin polymerization activities [23].

In addition to frequent potent activities, the 3-amino-1,2,4-triazole motif displays valuable inherent physicochemical-properties that improve overall solubility, bioavailability, or chemical stability of a molecule [21,25,26]. Moreover, the structural features of 3-amino-1,2,4-triazole enable it to mimic different functional groups, *e.g.* they are often considered as urea bioisostere, with enhanced solubility and decreased trend to form aggregates [26]. Besides, several clinically approved drugs with potent antiproliferative and antiangiogenic properties contain the diarylurea function [27], making,the 1,2,4-aminotriazole core an interesting and understudied pharmacophore.

In line with these observations, we investigated the anticancer potential of arylamino-3-pyrido-1,2,4-triazoles, which appears to be a particularly promising scaffold. Indeed, the fusion of structures I and II leads to a core with highly attractive steric and electronic features (Fig. 1**b**). This hybrid scaffold could display both antiproliferative and antiangiogenic activities, along with particularly favorable physicochemical properties for a drug. The molecular weight (237 g/mol), the partition coefficient (cLogP = 2.91), the number of hydrogen-bonds accepting sites (3–4), donating sites (2–3) and rotatable bonds (3) perfectly complies with the *Lipinski rules of three* standards and should lead to beneficial pharmacological properties for its derivatives. This arylamino-3-pyrido-1,2,4-triazole scaffold is also innovative as only one such series of compounds has been identified so far [23]. In this study, we revisited the synthesis of these 1,2,4-triazole compounds and prepared a series of analogs intending to further evaluate the synergism of the fusion of the two identified antiproliferative and antiangiogenic cores (Fig. 1c). In total, 25 new diaryl-1,2,4-triazole derivatives have been synthesized and fully characterized, as well as 14 benzoylthiourea intermediates. All compounds have been biologically tested to assess their activity on a panel of cancer cell lines, as well as on an endothelial cell line.

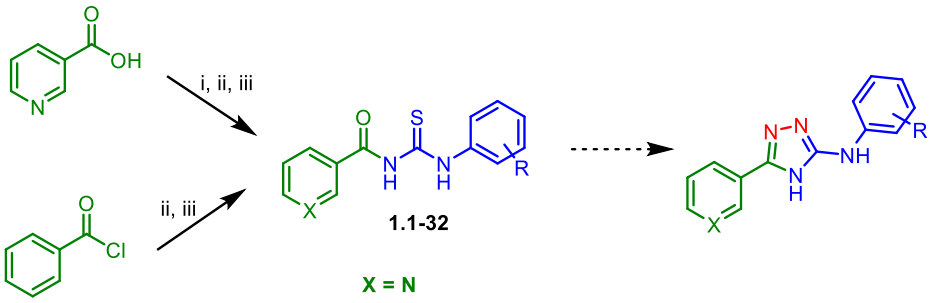
2. Results and discussion

2.1. Chemistry

The compounds synthesized can be divided into two groups depending on the nature of the cycle in position 5 of the triazole: 3-pyridyl (Series A) or phenyl (Series B) (Fig. 1**c**). The initial retrosynthetic analysis envisaged the synthesis of triazole compounds through the double condensation of hydrazine on *N*-(benzylcarbamothioyl)arylamide precursors **1** (Scheme 1). These precursors **1** could be obtained by the addition of diversely substituted anilines on arylcarbonyl isothiocyanates, which can be prepared from commercially available nicotinic acid or benzoyl chloride by adapting described procedures [31]. Thus, chlorination and treatment with ammonium isothiocyanate proceeded in quantitative yields [46], then the addition of variously substituted anilines provided *N*-(phenylcarbamothioyl)nicotinamides **1.1–10** and *N*-(phenylcarbamothioyl)benzamides **1.11–29** in generally high yields (77–96%) (Scheme 1) [35,47].

The formation of the 1,2,4-triazole by hydrazine condensation required a preliminary conditions screening (Table 1). We conducted a study of the effect of the solvent, the number of hydrazine equivalents, reaction temperature, and time on the model substrate **1.11**. Suitable conversion was found with the following conditions: addition of 3 equivalents of hydrazine hydrate at 0 °C in 1,4-dioxane and stirring at 20 °C for 24 h (Table 1**, Entry 7**). Using these conditions, *N*,3-diphenyl-1H-1,2,4-triazol-5-amines **2.1–10** were synthesized in good to moderate yields (Scheme 2). Unfortunately, the amide bond of nicotinamide precursors **1.1–10** revealed to be fragile when subjected to the high nucleophilicity of hydrazine. Despite all our efforts, cyclization attempts with hydrazine on our model substrate **1.1** always led to the cleavage of this bond yielding 1-phenylthioureas **3** as a major product (Scheme 2).

Consequently, an alternative synthetic pathway was developed for



X = N

1.1: R = H, 84%
1.2: R = 2-NO_2, 96%
1.3: R = 2-OEt, 77%
1.4: R = 2-Cl, 95%
1.5: R = 2-Br, 97%
1.6: R = 3-Br, 93%
1.7: R = 4-CH_3, 82%
1.8: R = 4-Cl, 91%
1.9: R = 3,4-di(Cl), 84%
1.10: R = 2,5-di(Cl), 97%

X = CH

1.11: R = H, 78%
1.12: R = 2-CH_3, 81%
1.13: R = 2-OH, 85%
1.14: R = 2-OEt, 77%
1.15: R = 2-NO_2, 92%
1.16: R = 2-Cl, 88%
1.17: R = 2-Br, 90%
1.18: R = 3-NO_2, 94%
1.19: R = 3-Br, 95%
1.20: R = 4-CH_3, 87%
1.21: R = 4-Cl, 96%
1.22: R = 3,4-di(CH_3), 87%
1.23: R = 2-OH-5-CH_3, 81%
1.24: R = 2-OH-6-CH_3, 81%
1.25: R = 2-OH-4-NO_2, 91%
1.26: R = 2-OH-4-Cl, 91%
1.27: R = 2-OMe-5-NO_2, 77%
1.28: R = 2-Cl-5-NO_2, 86%
1.29: R = 2,4-di(Cl), 94%
1.30 R = 2,5-di(Cl), 96%
1.31: R = 3,4-di(Cl), 97%
1.32: R = 3,5-di(Cl), 93%

Reagents and reaction conditions: (i) $SOCl_2$, reflux, 12 h; (ii) NH_4SCN, acetone, r.t., 2h; (iii) Anilines, acetone, r.t., 12 h; (iv) N_2H_4•H_2O, EtOH, reflux, 2 h

Scheme 1. Synthesis of 1,2,4-triazole precursors *N*-(phenylcarbamothioyl)nicotinamides **1.1-l0** and *N*-(phenylcarbamothioyl) benzamides **1.11–32**.

nicotinamide derivatives, which takes advantage of this weak amide bond, (Scheme 3). By using harsh reaction conditions, *N*-(phenylcarbamothioyl)benzamide precursors **1.11–32** could be hydrolyzed in hot alkaline solution (aq. 2 M NaOH, 85 °C) leading to substituted 1-phenylthioureas **3.1–12**. *S*-methylation of these compounds, followed by condensation with previously prepared nicotinohydrazide [48] allowed efficient triazole cyclization leading to the desired *N*-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-amines **4.1–12** (Scheme 4).

The modulation of the 1,2,4-triazole ring was studied *via* the synthesis of the analogs 1,3,4-oxadiazole **5**, 1,3,4-thiadiazole **6,** and 1*H*-pyrazole **7**. These compounds were obtained according to the sequences described in Scheme 4. The precursors of **5** and **6** were synthesized by the addition of nicotinohydrazide to phenyliso(thio)cyanate under reflux in 1,4-dioxane, followed by dehydrative cyclization in acidic medium ($POCl_3$ or H_2SO_4) yielding targeted oxadiazole **5** and thiadiazole **6**. The pyrazole analog **7** was prepared by the addition of 3-acetylpyridine enolate to phenyl isothiocyanate, followed by quenching with iodomethane, and cycle formation by hydrazine double condensation in 86% yield.

In summary, 3-pyridyl-containing analogs **4.1–12**, **5**, **6,** and **7** constitute Series A, aimed at studying both the influence of the modulations on the phenylamino moiety and of the 1,2,4-triazole. The phenyl-based triazole derivatives **2.1–10** and *N*-(phenylcarbamothioyl) benzamide precursors **1.11–32** are gathered in Series B which was produced to evaluate the replacement of the 3-pyridyl ring by phenyl and assess the activity potential of the (phenylcarbamothioyl)benzamide precursors. The related *N*-(phenylcarbamothioyl)nicotinamides **1.1–10** have not been evaluated due to their fragility towards nucleophiles (namely the instability of the amide bond), which suggests poor pharmacological properties for these molecules *in cellulo*.

2.2. Biological evaluation

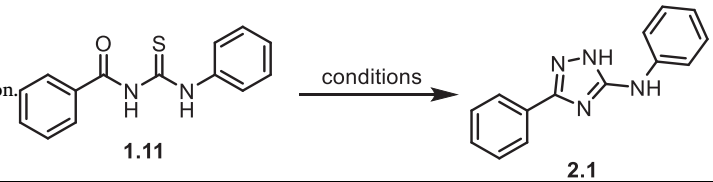
2.2.1. Antiproliferative activity on cancer cells

The new synthesized 3-(3-pyrido)-azole compounds of series A (**4.1–12, 5, 6,** and **7**) were subjected to cell viability assay on three aggressive cancer models for which no efficient and longstanding treatment is currently available, *i.e.* medulloblastoma, head and neck squamous cell carcinoma, and kidney cancer. Precisely, the 12 pyridotriazoles **4.1–12** as well as the imidazolo-, oxadiazolo- and thiadiazolo analogs **5–7** were screened against 6 cancer cell lines: Daoy and HD-MB03 human medulloblastoma cell lines; CAL27 and CAL33 head and neck squamous cell carcinoma cell lines; and A498 and 786-O kidney cancer cell lines. All the defined cells were treated with the new synthetic compounds for two days and XTT assays were used to measure cell metabolism (cell proliferative ability, by extension). The IC_{50} values (μM) are listed in Table 2. One of the tested compounds, **4.6**, bearing a 3-bromophenyl moiety, showed significant antiproliferative effects with IC_{50} values $<$ 15 μM on head and neck squamous head and neck and kidney cancers cells. On the other hand, the replacement of the 1,2,4-triazole by an imidazole, oxadiazole, or thiadiazole proved to be unfavorable.

The overall modest results observed prompted us to study another promising related fusion scaffold: *N*,3-diphenyl-1,2,4-triazol-5-amine (Scheme 2, **X = CH**), as well as some synthetic intermediates **1.11–29**. Only intermediates bearing a phenyl moiety were chosen for evaluation because of the lability of the amide bond observed for the pyridine analogues. Thus, these 10 new 3,5-diarylamino-1,2,4-triazoles **2.1–2.10** and the 14 *N*-benzoylthioureas **1.11–29**– consisting of compounds of series B – were subjected to biological evaluation of their antiproliferative potential under the same assay conditions on the six previously described cancer cell lines. The results for the 25 compounds

Table 1

Conditions survey for the 1,2,4-triazole formation.



Entry	Equiv. of $N_2H_4{\cdot}H_2O$	Solvent	Temperature (°C)	Time (h)	Conversion (%)
1	5	EtOH	reflux	24	< 5
2	5	EtOH	0 to r.t.	24	13
3	5	$CHCl_3$	0 to r.t.	24	28
4	5	1,4-dioxane	0 to r.t.	24	35
5	–	No solvent	0 to r.t.	24	19
6	3	$CHCl_3$	0 to r.t.	24	61
7	**3**	**1,4-dioxane**	**0 to r.t.**	**24**	**73**
8	3	1,4-dioxane	0 to r.t.	48	75
9	3	1,4-dioxane	30	24	30
10	5	1,4-dioxane	30	24	12

of series B are displayed in Table 3. Globally, the same structure-activity relationships were observed for the 3,5-diarylamino-1,2,4-triazole derivatives **2.1**–**2.10** with the presence of apolar substituents like a halogen or a nitro group that seemed to be favored. In particular, the 3-bromo substitution again provided the best compound of this series (**2.6**). Moreover, this clear beneficial effect of the 3-bromophenyl moiety was noted on all cell lines tested, with a slightly lower activity on medulloblastoma. This effect on cell lines of multiple origins predicts a successful use of this compound as a basis for a generic anti-tumoral therapeutic strategy, which remains to be thought of with the specificities of each pathology. Concerning the *N*-benzoylthioureas **1.11**–**29**, significant antiproliferative activity was only noticed for derivatives **1.13** and **1.25** with 2-hydroxy substituents on the HD-MB03 medulloblastoma cell line (IC_{50} = 5 μM) and for the 2-chloro-5-nitro derivative **1.28** on kidney cell lines A498 and 786-O. HD-MB03 cell line, derived from a highly aggressive form of medulloblastoma, seems to be much more sensitive to *N*-benzoylthioureas than its Daoy counterpart (less aggressive). This suggests that HD-MB03 cells are richer in *N*-benzoylthiourea targets than Daoy cells. This difference in sensitiveness was also demonstrated with the main reference treatment of this pathology, *i.e.* etoposide. Indeed, HD–MB03 cells were twice as sensitive to this compound as Daoy cells (Table 3), thus suggesting that HD-MB03 cells are more sensitive to therapeutic agents, as a whole, than Daoy cells. This confirms that medulloblastoma must be considered as a heterogeneous group of pathologies rather than one unique disease [49] and that a targeted therapeutic strategy might be the most promising path to follow.

Overall, the most compelling structures are the triazole compounds with a 3-bromophenyl moiety (**4.6** and **2.6**) since they show efficiency on all the tested cell lines. The *N*-benzoylthioureas with a 2-hydroxyphenyl moiety (**1.13** and **1.25**) also showed promising activities but further studies on these compounds are needed to understand the selectivity towards one specific cell line.

2.2.2. Antiangiogenic activity

The antimetabolic activity on non-cancer cells from the tumor microenvironment was tested in preliminary experiments. Tumor vasculature plays a capital part in tumor development, as blood vessels bring oxygen and nutrients, which contributes to tumor growth. Homeostasis of endothelial cell proliferation and permeability is then crucial. Affecting one or the other may lead to resorption of the tumor. Thus, we studied the antimetabolic activity of the newly prepared compounds of series B on an immortalized endothelial cell line (TIME), with the same XTT assay as a reflect of the antiproliferative, antiangiogenic activity of these compounds. The results, presented in Table 3, show that the triazole compound with a 3-bromophenyl moiety (**2.6**), one of the most efficient compounds on tumor cells, is also the most efficient in reducing the proliferation of their companion endothelial cells. It is noteworthy to mention the specific activity of compound **1.25** on the TIME endothelial cells and on two different types of medulloblastoma, but not on the other studied types of cancers. Similarly, product **1.28** is the only one which seems to act on two different models of renal clear cell carcinoma, a highly vascularized tumor, as well as on the TIME endothelial cells. 3D experiments of tumor cell proliferation and migration, vascular angiogenesis (spheroids) are likely to confirm these preliminary positive results. Besides, a highly favorable selectivity was observed for compounds **1.13** and **1.25** towards HD-MB03 medulloblastoma cells compared to TIME cells representative of healthy cells.

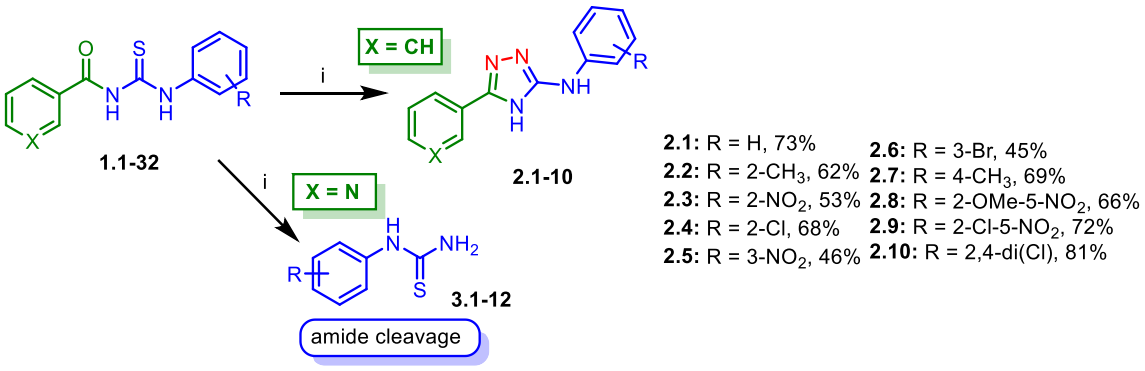
3. Material and methods

3.1. Chemistry

All the starting materials have been purchased from chemical providers Aldrich, Merck or Alfa Aesar and used without additional purification. All the solvents have been purchased from Sigma Aldrich and used without precursive distillation. Thin layer chromatography (TLC) have been performed on pre-coated silica gel plates (Kieselgel 60 F254, E. Merck, Germany) and visualized by UV lamp (wavelength 254 nm). NMR spectra have been recorded in DMSO-d_6 (^{1}H and ^{13}C NMR) and TFA-d_1 (13C NMR) on a Bruker AC 200 at 200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR, or on a Bruker AC 400 spectrometers at 400 MHz for 1H NMR and 101 MHz for ^{13}C NMR; δ is expressed in ppm related to TMS (0 ppm) as an internal standard. Splitting patterns are named as following: s (singlet), d (doublet), t (triplet), m (multiplet). Coupling constants (J) values are expressed in Hertz (Hz). Mass spectra (ESI-MS) have been recorded on a Bruker Daltonics Esquire 3000+, all the solid samples have been dissolved in methanol and pre-filtrated. The purity of compounds has been assayed by HPLC analysis on JASCO PU-2089, with Supelco analytical column Ascentis Express C18, 100 mm × 46 mm 5 μM. Eluent A: 99.9% water with 0.1% formic acid. Eluent B: 99.9% acetonitrile with 0.1% formic acid. Method 1: 25% B to 100% B for 13 min, 100% B for 5 min, from 100% B to 5% B for 1 min (19 min total).

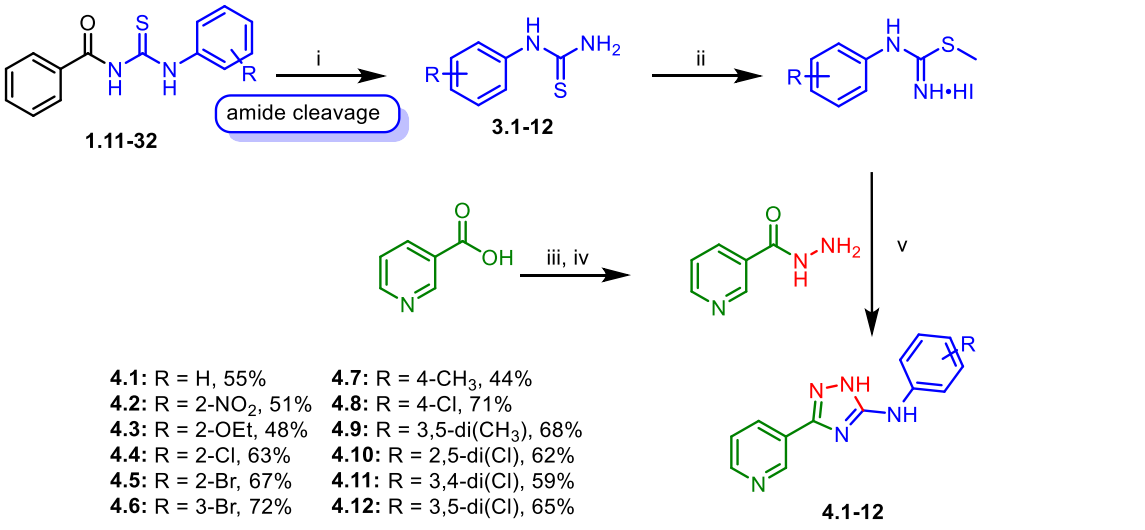
3.1.1. Synthetic procedures and characterizations

General Procedure A: To the solution of substituted *N*-(phenylcarbamothioyl)benzamide (1 equiv) in 1,4-dioxane (10 mL/1 mmol) at 0 °C, hydrazine hydrate (3 eq) was added. The reaction was stirred for 24–30 h at room temperature, the conversion was monitored by TLC



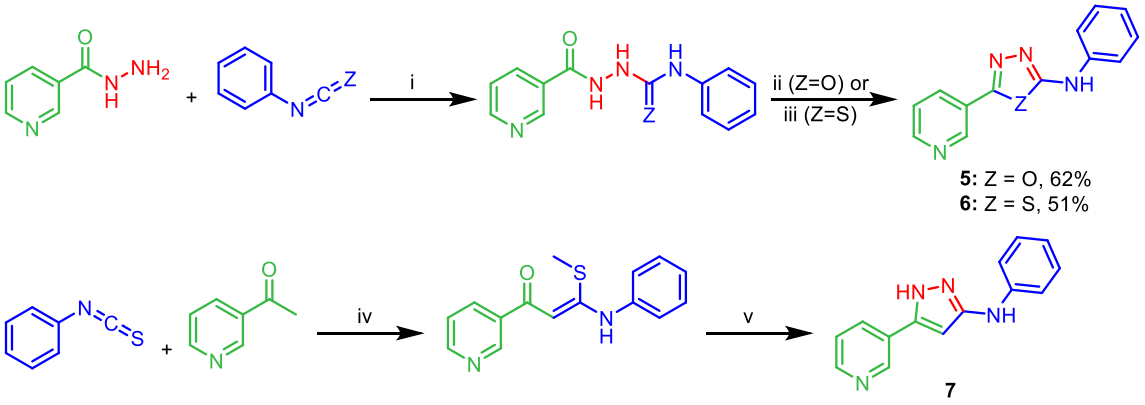
Reagents and reaction conditions: (i) N_2H_4•H_2O, 1,4-dioxane, 0 °C to r.t, 12 h.

Scheme 2. Synthesis of targeted *N*,3-diphenyl-1*H*-1,2,4-triazol-5-amines **2.1**–**10**; and cleavage of the amide bond to form 3-pyridyl derivatives **3.1**–**12**.



Reagents and reaction conditions: (i) $NaOH_{aq.}$, 85 °C, 1 h; (ii) Methyl Iodide, EtOH, reflux 1 h;(iii) MeOH, $SOCl_2$, reflux, 12 h; (iv) N_2H_4•H_2O, MeOH, reflux 4 h; (v) Pyridine, reflux, 12 h

Scheme 3. Synthesis of targeted *N*-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-amines compounds **4.1**–**12** by condensation of nicotinohydrazide [48] with phenyl-*S*-methylisothioureas.



Reagents and reaction conditions: (i) 1,4-Dioxane, reflux, 12 h; (ii) $POCl_3$, reflux, 2 h; (iii) H_2SO_4, 0 °C to r.t., 12 h; (iv) NaH, DMF, 0 °C, 1 h, then methyl Iodide, r.t., 1h; (v) N_2H_4•H_2O, EtOH, reflux 12 h.

Scheme 4. Synthesis of targeted 1,2,4-triazole analogues 1,3,4-oxadiazole **5**, 1,3,4-thiadiazole **6** and 1H-pyrazole **7**.

and LC-MS until the equilibrium was reached. The crude compound was purified by using column chromatography

General Procedure B: A mixture of substituted 1-phenylisothiouronium iodide (1 equiv) and nicotynohydrazide (1 equiv) in pyridine (10 mL/1 mmol) was refluxed overnight. After the reaction was completed (TLC monitoring) the solution was poured into

Table 2
Antiproliferative activities of synthetic compounds **4.1–12, 5, 6,** and **7** of series A.

Compounds	IC_{50} (μM)					
	Daoy medulloblastoma	HD-MB03 medulloblastoma	CAL27 head and neck	CAL33 head and neck	A498 kidney	786-O kidney
4.1	> 50	> 50	> 50	> 50	> 50	> 50
4.2	> 50	> 50	> 50	50 ± 1.1	> 50	> 50
4.3	> 50	> 50	> 50	> 50	40 ± 4.1	> 50
4.4	> 50	> 50	> 50	> 50	> 50	> 50
4.5	> 50	> 50	> 50	50 ± 4.3	50 ± 4.2	> 50
4.6	> 50	32 ± 2.8	12 ± 2.0	10 ± 1.4	10 ± 1.7	15 ± 1.3
4.7	> 50	> 50	> 50	> 50	> 50	> 50
4.8	> 50	> 50	> 50	30 ± 2.7	40 ± 3.1	> 50
4.9	> 50	> 50	40 ± 3.2	> 50	> 50	> 50
4.10	> 50	> 50	> 50	50 ± 2.2	50 ± 3.1	> 50
4.11	> 50	> 50	> 50	45 ± 4.2	25 ± 3.2	> 50
4.12	> 50	> 50	> 50	35 ± 2.8	40 ± 2.9	> 50
5	> 50	> 50	> 50	> 50	> 50	> 50
6	> 50	> 50	> 50	> 50	> 50	> 50
7	> 50	> 50	> 50	> 50	> 50	> 50
carboplatine	24.3 ± 6.1	46.3 ± 7.8	N/A	N/A	N/A	N/A
cisplatin	N/A	N/A	2.0 ± 0.2	3.5 ± 0.3	N/A	N/A
sunitinib	N/A	N/A	N/A	N/A	4.0 ± 0.3	3.5 ± 0.3

All values are the mean of 3 experiments ± SD.

the water (50 mL/1 mmol) and extracted with ethyl acetate (3 × 25 mL). Organic layer was collected, washed with brine, dried with $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude compound was purified by using column chromatography.

Compounds **1.1–32** were synthesized by following the previously described procedures [28–39]. The NMR spectral data of compounds **1.1–32** were in accordance with those reported in the literature.

N,5-diphenyl-1H-1,2,4-triazol-3-amine [40] (**2.1**) Synthesized following the general procedure A using *N*-(phenylcarbamothioyl)benzamide (0.256 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (172 mg, 73%). ^{1}H NMR (400 MHz, TFA-*d1*): *δ* 7.75 (d, *J* = 7.8 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.50 (dt, *J* = 12.6, 7.6 Hz, 4H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.35 (dd, *J* = 7.4, 1.7 Hz, 2H). ^{13}C NMR (101 MHz, TFA-*d1*): *δ* 153.73, 152.96, 135.69, 135.65, 132.82 (2C), 131.81 (2C), 131.53, 128.68 (2C), 126.48 (2C), 123.77. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{13}N_4^+$, 237.11; Found: 237.27. HPLC (λ_{280}): Purity 100.0%; t_R: 1.092 min (method 1).

5-phenyl-N-(o-tolyl)-4H-1,2,4-triazol-3-amine (**2.2**) Synthesized following the general procedure A using *N*-(2-tolylcarbamothioyl)benzamide (0.270 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (155 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 13.70 (s, 0.5H), 12.35 (s, 0.5H), 8.47 (s, 0.5H), 8.05–7.76 (m, 3.5H), 7.63–7.29 (m, 3H), 7.15 (d, *J* = 11.2 Hz, 2H), 6.87 (d, *J* = 45.9 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): *δ* 161.21, 158.59, 154.24, 152.44, 140.20, 138.90, 131.91, 130.33, 129.93, 128.95, 128.81, 128.57, 127.43, 127.08, 126.51, 126.33, 125.63, 122.01, 120.22, 118.81, 117.37, 17.99. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{15}H_{15}N_4^+$, 251.13; Found: 251.27. HPLC (λ_{280}): Purity 100.0%; t_R: 1.125 min (method 1).

N-(2-nitrophenyl)-5-phenyl-4H-1,2,4-triazol-3-amine (**2.3**) Synthesized following the general procedure A using *N*-((2-nitrophenyl)carbamothioyl)benzamide (0.301 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a yellow powder (149 mg, 53%). ^{1}H NMR (200 MHz, DMSO-d_6): *δ* 14.13 (s, 1H), 10.16 (s, 1H), 8.60 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.11–7.88 (m, 2H), 7.73 (t, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 6.0 Hz, 3H), 7.04 (t, *J* = 7.8 Hz, 1H). ^{13}C NMR (101 MHz, TFA-*d1*): *δ* 153.00, 152.36, 139.68, 138.54, 137.43, 135.89, 132.49 (2C), 129.20 (2C), 128.84, 126.69, 122.74, 120.63. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{12}N_5O_2^+$, 282.10; Found: 282.07. HPLC (λ_{280}): Purity 99.1%; t_R: 1.167 min (method 1).

N-(2-chlorophenyl)-5-phenyl-1H-1,2,4-triazol-3-amine [40] (**2.4**) Synthesized following the general procedure A using *N*-((2-chlorophenyl)carbamothioyl)benzamide (0.291 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (184 mg, 68%). ^{1}H NMR (400 MHz, TFA-*d1*): *δ* 7.86–7.78 (m, 2H), 7.67–7.60 (m, 1H), 7.54 (td, *J* = 7.3, 6.2, 3.0 Hz, 4H), 7.44–7.34 (m, 2H). ^{13}C NMR (101 MHz, TFA-*d1*): *δ* 153.64, 152.76, 135.88, 133.25, 133.12, 132.62, 132.52, 131.84 (2C), 130.83, 128.85 (2C), 128.67, 123.33. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{12}ClN_4^+$, 271.07; Found: 271.20. HPLC (λ_{280}): Purity 99.4%; t_R: 1.175 min (method 1).

N-(3-nitrophenyl)-5-phenyl-4H-1,2,4-triazol-3-amine (**2.5**) [41] Synthesized following the general procedure A using *N*-((3-nitrophenyl) carbamothioyl)benzamide (0.301 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a yellow powder (129 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 13.97 (s, 1H), 9.96 (s, 1H), 8.71 (t, *J* = 2.3 Hz, 1H), 7.92 (dd, *J* = 46.7, 7.8 Hz, 3H), 7.76–7.39 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d_6): *δ* 160.03, 152.55, 148.48, 143.23, 130.20, 129.90, 129.10 (2C), 127.10, 125.82 (2C), 122.00, 113.47, 109.36. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{12}N_5O_2^+$, 282.10; Found: 282.07. HPLC (λ_{280}): Purity 99.3%; t_R: 1.108 min (method 1).

N-(3-bromophenyl)-5-phenyl-1H-1,2,4-triazol-3-amine (**2.6**) Synthesized following the general procedure A using *N*-((3-bromophenyl)carbamothioyl)benzamide (0.335 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (142 mg, 45%). ^{1}H NMR (400 MHz, TFA-*d1*): *δ* 7.72 (dd, *J* = 7.3, 1.6 Hz, 2H), 7.62–7.56 (m, 1H), 7.54–7.43 (m, 4H), 7.32–7.22 (m, 2H). 13C NMR (101 MHz, TFA-*d1*): *δ* 153.79, 152.82, 137.31, 135.91, 134.12, 133.76, 131.88 (2C), 128.94, 128.80 (2C), 126.05, 124.33, 123.39. LCMS (*m/z*): $[M+H]^+$ calc. for $C_{14}H_{12}BrN_4^+$, 315.02; Found: 315.15. HPLC (λ_{280}): Purity 100.00%%; t_R: 1.092 min (method 1).

5-phenyl-N-(p-tolyl)-1H-1,2,4-triazol-3-amine [40] (**2.7**) Synthesized following the general procedure A using *N*-(4-tolylcarbamothioyl)benzamide (0.270 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (173 mg, 69%). ^{1}H NMR (400 MHz, TFA-*d1*): *δ* 7.70 (d, *J* = 7.6 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.32–7.10 (m, 4H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, TFA-*d1*): *δ* 153.68, 153.16, 142.97, 135.73, 133.48 (2C), 133.06, 131.92 (2C), 128.96 (2C), 126.95 (2C), 124.08, 21.74. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{15}H_{15}N_4^+$, 251.13; Found: 251.27. HPLC (λ_{280}): Purity 99.1%; t_R: 1.108 min (method 1).

N-(2-methoxy-5-nitrophenyl)-5-phenyl-1H-1,2,4-triazol-3-amine (**2.8**) Synthesized following the general procedure A using *N*-((2-methoxy-5-

Table 3
Antiproliferative activities of synthetic compounds of series B (**compounds 2.1–10** and some of **1.11–29**).

Compounds	IC_{50} (μM)						
	TIME endothelial cells	Daoy medulloblastoma	HD-MB03 medulloblastoma	CAL27 head and neck	CAL33 head and neck	A498 kidney	786-O kidney
2.1	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.2	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.3	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.4	> 50	> 50	> 50	> 50	45 ± 3.4	> 50	> 50
2.5	> 50	35 ± 1.9	35 ± 2.3	40 ± 4.1	40 ± 3.8	30 ± 3.4	> 50
2.6	15 ± 1.8	40 ± 3.4	40 ± 2.5	15 ± 2.4	10 ± 0.8	15 ± 1.8	15 ± 1.4
2.7	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.8	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.9	> 50	> 50	> 50	> 50	> 50	> 50	> 50
2.10	> 50	> 50	34	35 ± 3.9	20 ± 2.4	25 ± 2.7	20 ± 1.9
1.11	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.12	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.13	> 50	> 50	5 ± 1.7	> 50	> 50	> 50	> 50
1.15	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.16	> 50	> 50	> 50	> 50	35 ± 2.7	> 50	> 50
1.18	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.19	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.23	20 ± 2.0	> 50	> 50	> 50	> 50	> 50	> 50
1.24	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.25	20 ± 1.5	20 ± 3.4	5 ± 1.3	> 50	> 50	> 50	> 50
1.26	> 50	> 50	> 50	> 50	> 50	> 50	> 50
1.27	20 ± 2.8	> 50	> 50	> 50	> 50	> 50	> 50
1.28	20 ± 2.2	> 50	> 50	> 50	> 50	10 ± 3.0	30 ± 3.7
1.29	> 50	> 50	> 50	> 50	> 50	> 50	> 50
carboplatine	N/A	24.3 ± 6.1	46.3 ± 7.8	N/A	N/A	N/A	N/A
cisplatine	N/A	N/A	N/A	2.0 ± 0.2	3.5 ± 0.3	N/A	N/A
sunitinib	N/A	N/A	N/A	N/A	N/A	4.0 ± 0.3	3.5 ± 0.3

All values are the mean of 3 experiments ± SD.

nitrophenyl)carbamothioyl)benzamide (0.331 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a yellow powder (205 mg, 66%). ^{1}H NMR (400 MHz, TFA-*d1*): δ 8.79 (d, *J* = 2.6 Hz, 1H), 8.23 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.95–7.89 (m, 2H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 9.2 Hz, 1H), 4.09 (s, 3H). ^{13}C NMR (101 MHz, TFA-*d1*): δ 158.30, 152.90, 152.69, 142.96, 136.74, 132.18 (2C), 129.16 (2C), 128.04, 125.21, 121.66, 118.23, 112.93, 58.38. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{15}H_{14}N_5O_3^+$, 312.11; Found: 312.13. HPLC (λ_{280}): Purity 99.7%; t_R: 1.100 min (method 1).

N-(2-chloro-5-nitrophenyl)-5-phenyl-1H-1,2,4-triazol-3-amine (**2.9**) Synthesized following the general procedure A using *N*-((2-chloro-5-nitrophenyl)carbamothioyl)benzamide (0.335 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a yellow powder (227 mg, 72%). ^{1}H NMR (400 MHz, TFA-*d1*): δ 8.98 (d, *J* = 2.5 Hz, 1H), 8.07 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.89–7.83 (m, 2H), 7.70 (dd, *J* = 14.1, 8.3 Hz, 2H), 7.62 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, TFA-*d1*): δ 153.31, 152.88, 149.02, 137.08, 136.64, 134.81, 133.16, 132.25 (2C), 129.17 (2C), 122.89, 120.97, 118.39. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{11}ClN_5O_2^+$, 316.06; Found: 316.13. HPLC (λ_{280}): Purity 99.1%; t_R: 1.158 min (method 1).

N-(2,4-dichlorophenyl)-5-phenyl-1H-1,2,4-triazol-3-amine (**2.10**) Synthesized following the general procedure A using N-((2,4-dichlorophenyl)carbamothioyl)benzamide (0325 g, 1 mmol) and hydrazine hydrate (0.146 mL, 3 mmol) to afford the title compound as a white powder (247 mg, 81%). ^{1}H NMR (400 MHz, TFA-*d1*): δ 7.77–7.70 (m, 2H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.55–7.44 (m, 4H), 7.33 (dd, *J* = 8.6, 2.3 Hz, 1H). ^{13}C NMR (101 MHz, TFA-*d1*): δ 153.78, 152.98, 138.21, 136.10, 133.07, 132.99, 132.01, 131.94 (2C), 131.08, 129.06, 128.83 (2C), 122.98. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{11}Cl_2N_4^+$, 305.04; Found: 305.20. HPLC (λ_{280}): Purity 99.7%%; t_R: 1.150 min (method 1).

N-phenyl-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.1**) Synthesized following the general procedure B using 2-methyl-1-phenylisothiouronium iodide (0.294 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). Beige powder (130 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.93 (s, 0.5H), 12.86 (s, 0.5H), 9.43 (d, *J* = 83.3 Hz, 1H), 9.16 (s, 1H), 8.64 (s, 1H), 8.30 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.59 (m, *J* = 8.1 Hz, 3H), 7.27 (s, 2H), 6.86 (d, *J* = 30.5 Hz, 1H). ^{13}C NMR (101 MHz, TFA-*d1*): δ 154.09, 147.08, 146.36, 145.56, 142.11, 135.31, 133.035 (2C), 132.10, 130.92, 128.24, 126.865 (2C). LC-MS (*m/z*): $[M+H]^+$ calc. for $C_{13}H_{11}N_5^+$, 238.11; Found: 238.27. HPLC (λ_{280}): Purity 99.5%; t_R: 1.475 min (method 1).

N-(2-nitrophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.2**) Synthesized following the general procedure B using 2-methyl-1-(2-nitrophenyl)isothiouronium iodide (0.339 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as an orange powder (144 mg, 51%). ^{1}H NMR (400 MHz, TFA-*d1*): δ 9.71 (d, *J* = 1.9 Hz, 1H), 9.38 (dt, *J* = 8.4, 1.6 Hz, 1H), 9.15 (d, *J* = 5.7 Hz, 1H), 8.46 (ddd, *J* = 8.4, 3.6, 2.2 Hz, 2H), 7.99 (dtd, *J* = 15.5, 8.2, 1.5 Hz, 2H), 7.71–7.64 (m, 1H). ^{13}C NMR (400 MHz, TFA-*d1*): δ 153.64, 149.13, 147.23, 145.44, 142.87, 142.25, 138.96, 132.70, 130.89, 130.25, 129.20, 129.00, 125.90. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}N_6O_2^+$, 283.09; Found: 283.13. HPLC (λ_{280}): Purity 100.0%; t_R: 1.192 min (method 1).

N-(2-ethoxyphenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.3**) Synthesized following the general procedure B using 1-(2-ethoxyphenyl)-2-methylisothiouronium iodide (0.338 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (3:7). Beige powder (135 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.03 (s, 0.3H), 12.44 (s, 0.7H), 9.17 (d, *J* = 2.2 Hz, 1H), 8.64 (d, *J* = 30.6 Hz, 1H), 8.42 (s, 0.7H), 8.31 (dt, *J* = 8.1, 2.0 Hz, 1.7H), 8.16 (s, 0.3H), 7.50 (s, 1.3H), 6.98 (dd, *J* = 31.1, 9.0 Hz, 3H), 4.14 (q, *J* = 6.4 Hz, 2H), 1.41 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 156.40, 153.77, 149.53, 146.72, 146.50, 132.81, 129.44, 127.52, 123.82, 121.22, 120.72, 116.69, 111.76, 63.91, 14.71. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{15}H_{16}N_5O^+$, 282.13; Found: 282.27. HPLC (λ_{280}): Purity 99.5%; t_R: 1.258 min (method 1).

N-(2-chlorophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.4**) Synthesized following the general procedure B using 1-(2-chlorophenyl)-2-methylisothiouronium iodide (0.328 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). White powder (171 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.12 (s, 0.5H), 12.61 (s, 0.5H), 9.19 (d, J = 2.1 Hz, 1H), 8.89 (s, 0,5H), 8.62 (d, J = 4.8 Hz, 1H), 8.31 (dt, J = 7.9, 2.0 Hz, 2,5H), 7.61–7.27 (m, 3H), 6.97 (t, J = 7.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 156.45, 153.32, 149.91, 146.84, 137.16, 133.02, 129.41, 127.94, 127.45, 123.91, 122.25, 120.95, 118.83. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}ClN_5^+$, 272.07; Found: 272.27. HPLC (λ_{280}): Purity 98.9%; t_R: 1.275 min (method 1).

N-(2-bromophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.5**) Synthesized following the general procedure B using 1-(2-bromophenyl)-2-methylisothiouronium iodide (0.372 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). White powder (211 mg, 67%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.11 (s, 0.5H), 12.69 (s, 0.5H), 9.18 (d, J = 2.2 Hz, 1H), 8.88–8.47 (m, 2H), 8.36–8.14 (m, 2H), 7.61 (dd, J = 8.0, 1.5 Hz, 1H), 7.57–7.45 (m, 1H), 7.44–7.33 (m, 1H), 6.92 (t, J = 7.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 156.53, 153.52, 149.90, 146.82, 138.32, 133.02, 132.69, 128.55, 127.27, 123.91, 123.18, 119.36, 111.84. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}BrN_5^+$, 316.02; Found: 316.13. HPLC (λ_{280}): Purity 98.0%; t_R: 1.275 min (method 1).

N-(3-bromophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.6**) Synthesized following the general procedure B using 1-(3-bromophenyl)-2-methylisothiouronium iodide (0.372 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). Beige powder (227 mg, 72%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.04 (s, 0.5H), 13.06 (s, 0.5H), 9.65 (s, 1H), 9.15 (dd, J = 5.6, 2.2 Hz, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.29 (dt, J = 8.0, 2.0 Hz, 1H), 7.91 (t, J = 2.0 Hz, 1H), 7.67–7.41 (m, 2H), 7.21 (t, J = 8.2 Hz, 1H), 7.00 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.46, 150.79, 150.27, 146.85, 143.46, 133.22, 130.69, 124.05, 123.35, 121.89, 121.63, 117.97, 114.81. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}BrN_5^+$, 316.02; Found: 316.33. HPLC (λ_{280}): Purity 96.2%; t_R: 1.233 min (method 1).

5-(pyridin-3-yl)-N-(p-tolyl)-1,2,4-triazol-3-amine (**4.7**) Synthesized following the general procedure B using 2-methyl-1-(p-tolyl)isothiouronium iodide (0.308 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (6:4). White powder (110 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.15 (s, 0.4H), 12.70 (s, 0.6H), 9.18 (m, J = 2.1 Hz, 1.6H), 8.63 (s, 1H), 8.52–8.02 (m, 2.4H), 7.74–7.24 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 153.81, 147.08, 146.89, 145.48, 142.13, 139.72, 134.50, 133.39, 131.38, 131.16, 130.85, 130.42, 128.19. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{10}Cl_2N_5^+$, 306.03; Found: 306.27. HPLC (λ_{280}): Purity 98.2%; t_R: 1.383 min (method 1).

N-(4-chlorophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.8**) Synthesized following the general procedure B using 1-(4-chlorophenyl)-2-methylisothiouronium iodide (0.329 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). Beige powder (192 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.99 (s, 0.5H), 12.95 (s, 0.5H), 9.62 (d, J = 67.9 Hz, 1H), 9.15 (dd, J = 8.0, 2.3 Hz, 1H), 8.65 (s, 1H), 8.29 (dt, J = 7.9, 2.0 Hz, 1H), 7.70–7.43 (m, 3H), 7.32 (d, J = 13.3 Hz, 2H). ^{13}C NMR (101 MHz, TFA-*d1*): δ 153.91, 147.05, 146.54, 145.50, 142.11, 138.59, 133.71, 133.14 (2C), 130.85, 128.16 (2C). LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}ClN_5^+$, 272.07; Found: 272.33. HPLC (λ_{280}): Purity 99.4%; t_R: 1.233 min (method 1).

N-(3,5-dimethylphenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.9**) Synthesized following the general procedure B using 1-(3,5-dimethylphenyl)-2-methylisothiouronium iodide (0.322 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). White powder (180 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.90 (s, 0.5H), 12.85 (s, 0.5H), 9.40 (s, 0.5H), 9.17 (d, J = 6.6 Hz, 1.5H), 8.75–8.51 (m, 1H), 8.29 (d, J = 8.5 Hz, 1H), 7.53 (dt, J = 36.4, 6.5 Hz, 1H), 7.19 (d, J = 35.4 Hz, 2H), 6.50 (d, J = 46.8 Hz, 1H), 2.23 (d, J = 8.0 Hz, 6H). ^{13}C NMR (50 MHz, TFA-*d1*): δ 154.15, 147.09, 146.28, 145.58, 144.05, 142.12, 135.06, 133.72, 130.97 (2C), 128.33, 124.23 (2C), 21.76 (2C). LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{15}H_{16}N_5^+$, 266.14; Found: 266.27. HPLC (λ_{280}): Purity 99.7%; t_R: 1.250 min (method 1).

N-(2,5-dichlorophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.10**) Synthesized following the general procedure B using 1-(2,5-dichlorophenyl)-2-methylisothiouronium iodide (0.363 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (1:1). Beige powder (189 mg, 62%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 14.25 (s, 0.5H), 12.66 (s, 0.5H), 9.16 (dd, J = 2.3, 0.9 Hz, 1H), 8.77–8.17 (m, 4H), 7.50 (dd, J = 20.2, 7.8 Hz, 2H), 6.99 (d, J = 8.0 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 150.65, 150.28, 146.84, 138.51 (2C), 133.16, 132.29, 130.69, 124.08, 121.29 (2C), 119.04, 117.51. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{10}Cl_2N_5^+$, 306.03; Found: 306.27. HPLC (λ_{280}): Purity 95.6%; t_R: 1.358 min (method 1).

N-(3,4-dichlorophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.11**) Synthesized following the general procedure B using 1-(3,4-dichlorophenyl)-2-methylisothiouronium iodide (0.363 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (7:3). White powder (180 mg, 59%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.10 (s, 0.7H), 13.13 (s, 0.3H), 9.87 (d, J = 60.4 Hz, 1H), 9.15 (s, 1H), 8.77–8.55 (m, 1H), 8.29 (d, J = 7.9 Hz, 1H), 7.94 (s, 1H), 7.67–7.39 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.21, 150.89, 150.40, 146.87, 142.08, 133.28, 131.03, 130.54, 124.09, 123.28, 120.14, 116.67, 116.12. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{10}Cl_2N_5^+$, 306.03; Found: 306.27. HPLC (λ_{280}): Purity 98.5%; t_R: 1.325 min (method 1).

N-(3,5-dichlorophenyl)-5-(pyridin-3-yl)-1,2,4-triazol-3-amine (**4.12**) Synthesized following the general procedure B using 1-(3,5-dichlorophenyl)-2-methylisothiouronium iodide (0.363 g, 1 mmol) and nicotynohydrazide (0.137 g, 1 mmol) to afford the title compound as a mixture of 1-H triazole and 2-H triazole tautomers (6:4). White powder (198 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.15 (s, 0.4H), 12.70 (s, 0.6H), 9.18 (m, J = 2.1 Hz, 1.6H), 8.63 (s, 1H), 8.52–8.02 (m, 2.4H), 7.74–7.24 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 153.81, 147.08, 146.89, 145.48, 142.13, 139.72, 134.50, 133.39, 131.38, 131.16, 130.85, 130.42, 128.19. LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{10}Cl_2N_5^+$, 306.03; Found: 306.27. HPLC (λ_{280}): Purity 98.2%; t_R: 1.383 min (method 1).

N-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine [42] (**5**) A solution of 2-nicotinoyl-*N*-phenylhydrazinecarboxamide (1.19 g, 5 mmol) in $POCl_3$ (5 mL) was refluxed for 2 h. After cooling to room temperature, the reaction mixture was poured into a cold water (200 mL). After stirring for 10 min the precipitated product was collected by filtration, washed with water and Et_2O, and dried under reduced pressure yielding to a pure product as a white powder (0.74 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.79 (s, 1H), 9.07 (dd, J = 2.3, 0.9 Hz, 1H), 8.74 (dd, J = 4.8, 1.6 Hz, 1H), 8.26 (ddd, J = 8.0, 2.3, 1.7 Hz, 1H), 7.65–7.58 (m, 3H), 7.41–7.34 (m, 2H), 7.03 (tt, J = 7.4, 1.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 160.30, 155.96, 151.59, 146.33, 138.52, 133.18, 129.21 (2C), 124.40, 122.15, 120.46, 117.23 (2C). LC-MS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}N_4O^+$, 239,09; Found: 239,13. HPLC (λ_{280}): Purity 98.4%; t_R: 3.617 min (method 1).

N-phenyl-5-(pyridin-3-yl)-1,3,4-thiadiazol-2-amine [43] (**6**) To a dry 2-nicotinoyl-*N*-phenylhydrazinecarbothioamide (0.408 g, 1.5 mmol) H_2SO_4 (15 mL, 30 mmol) was added slowly with syringe at 0 °C and the stirring solution was left overnight at room temperature under argon atmosphere. After the reaction was completed as monitored by TLC the reaction mixture was poured into a cold water (200 mL) and the pH was adjusted to 7 with NaOH solution. White precipitate was separated by

filtration and purified by column chromatography yielding to a pure product as a white powder (130 mg, 51%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.66 (s, 1H), 9.05 (dd, J = 2.3, 0.9 Hz, 1H), 8.67 (dd, J = 4.8, 1.6 Hz, 1H), 8.26 (ddd, J = 8.0, 2.4, 1.6 Hz, 1H), 7.72–7.61 (m, 2H), 7.56 (ddd, J = 8.0, 4.8, 0.9 Hz, 1H), 7.45–7.31 (m, 2H), 7.04 (t, J = 7.2 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 164.70, 154.59, 150.86, 147.34, 140.38, 134.04, 129.18 (2C), 126.57, 124.23, 122.27, 117.64 (2C). LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{13}H_{11}N_4S^+$, 255,07; Found: 255.13. HPLC (λ_{280}): Purity 100.0%; t_R: 1.175 min (method 1).

N-phenyl-5-(pyridin-3-yl)-1H-pyrazol-3-amine [44] (**7**) To a solution of 3-(methylthio)-3-(phenylamino)-1-(pyridin-3-yl)propan-1-one (1.18 g, 5 mmol) in isopropanol (30 mL) hydrazine hydrate (1.25 g, 25 mmol) was added and the reaction mixture was refluxed overnight. The solvent was evaporated under reduced pressure to yield pure product as a light pink powder (1.01 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.63 (s, 1H), 8.98 (d, J = 2.2 Hz, 1H), 8.56–8.45 (m, 2H), 8.12 (dt, J = 8.0, 2.0 Hz, 1H), 7.46 (dd, J = 8.0, 4.8 Hz, 1H), 7.30 (d, J = 7.7 Hz, 2H), 7.19 (dd, J = 8.6, 7.1 Hz, 2H), 6.73 (t, J = 7.2 Hz, 1H), 6.42 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 151.87, 148.80, 146.26, 143.88, 139.84, 132.28, 128.93 (2C), 126.36, 123.97, 118.31, 114.89 (2C), 91.67. LCMS *(m/z)*: $[M+H]^+$ calc. for $C_{14}H_{13}N_4^+$, 237,11; Found: 237.13. HPLC (λ_{280}): Purity 97.4%; t_R: 1.833 min (method 1).

3.2. Cell culture

The human medulloblastoma DAOY (Sonic Hedgehog subgroup) and HD-MB03 (Group 3) cell lines were obtained from Dr. Celio POUPONNOT (Institut Curie, Paris, FRANCE). They were cultured at 37 °C, in a 5% CO_2 incubator, in Eagle's Minimal Essential Medium (MEMα; Gibco® Life Technologies, Villebon-sur-Yvette, FRANCE) supplemented with 10% fetal calf serum (D. Dutscher, Brumath, FRANCE), GlutaMAX (Invitrogen®, Carlsbad, CA, USA) and 1 mM sodium pyruvate (Gibco®). Two human Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines, CAL33 and CAL27, were provided through a Material Transfer Agreement with the Oncopharmacology Laboratory, Centre Antoine Lacassagne (CAL), where they had initially been isolated [45]. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco®) supplemented with 7% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA). Two human Clear Cell Renal Cell Carcinoma (ccRCC; 786-O and A498) cell lines were purchased from the American Tissue Culture Collection (ATCC, Molsheim, FRANCE) and cultured in DMEM with 7% fetal bovine serum.

3.3. Cytotoxicity measurement (XTT)

Principle of the measurement: The assay is based on the cleavage of the tetrazolium salt 2,3-Bis-(2-méthoxy-4-nitro-5-sulfophényle)–2*H*-tétrazolium-5-carboxanilide (XTT, Sigma-Aldrich®, Saint-Quentin-Fallavier, FRANCE), in the presence of an electron-coupling reagent, producing a soluble formazan salt. This conversion only occurs in viable (metabolically active) cells. The number of viable cells is directly correlated with the amount of orange formazan by measuring the absorbance at 450 nm of the dye on a spectrophotometer.

3.3.1. Medulloblastoma and endothelial cells

5,000 DAOY cells and 50,000 HD-MB03 cells (for tumor cells), 20,000 TIME cells (for endothelial cells) were incubated in a 96-well plate in triplicate with two different concentrations of inhibitors (5 and 20 μM), for 48 h, in a total volume of 100 μl. A blank without cells was produced. At the indicated time, 50 μl of XTT reagent were added to each well. After incubation at 37 °C for 30 min − 1 h30 (depending on the cell type), the absorbance of the produced formazan was measured at 450 nm using the Promega GLOMAX®-Multi + detection spectrophotometer. The gross relative effect of each new compound on each cell line was assessed by normalizing the mean optical density (3 determinations of a product-containing well), at each concentration, by the mean optical density obtained for the same cell line without any added agent.

3.3.2. Head and neck and kidney cancer cells.

Cells (5 × 10^3 cells/100 μl) were incubated in a 96-well plate with different effectors for 48 h. 50 μl of XTT reagent were added to each well. The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolically active cells. Absorbance of the formazan product, reflecting cell viability, was measured at 490 nm. Each assay was performed in triplicate.

4. Conclusions

Two series of new synthetic azole compounds were synthesized and evaluated for their *in vitro* antiproliferative activity against different aggressive cancer models (two medulloblastoma, two head and neck squamous cells and two kidney cancer cell lines) for which no efficient and longstanding treatment is currently available. These derivatives consist of arylamino-1,2,4-triazoles, substituted in position 5 of the triazole either by a 3-pyridyl (series A) or a phenyl (series B). To the series B were also added some *N*-benzoylthiourea precursors. The synthetic pathway for series A derivatives was adapted by taking advantage of the amide bond weakness of the corresponding nicotinamide thiourea precursors. The structure-activity relationship allowed a first stepwise optimization. The comparison of the corresponding IC_{50} values showed i) the necessity to keep the 1,2,4-triazole core towards its replacement by other azoles, ii) a clear beneficial effect of the 3-bromophenylamino moiety in position 3 of the triazole for both Series A (compound **4.6**) and Series B (compound **2.6**), on all cell lines tested. Moreover, preliminary results on the antimetabolic activity of the compounds of series B were obtained on an immortalized endothelial cell line (TIME), demonstrating the antiangiogenic activity of these compounds. Altogether, these results show that the 5-aryl-3-phenylamino-1,2,4-triazole structure possesses fair anticancer activities and that derivatives with a 3-bromophenyl moiety seem to be the most promising ones.

Declaration of Competing Interest

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104271.

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