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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Novel 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives: synthesis, in vitro antitumor activity and computational studies

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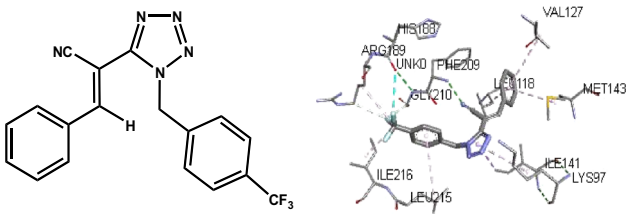
**Abstract** This work describes the two-step synthesis of new series of 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives (**6a–k**) starting from substituted benzyl halides (**5a–k**) and 3-phenyl-2-(1*H*-tetrazol-5-yl)acrylonitrile (**4**). Initially, compound 4 was synthesized using benzaldehyde, malononitrile and sodium azide. All the synthesized compounds were obtained in good yields and were characterized using $^{1}$H NMR, $^{13}$C NMR, FTIR and HRMS spectral data. The new compounds (6a–k) were evaluated for their potential in vitro antitumor activity against four human cancer cell lines (MCF-7, $CaCO_2$, HeLa and $SkBr_3$) by MTT assay. The most potent compounds **6b**, **6h** and **6j** show good activity ($IC_{50}$ values) relative to 5-fluorouracil, with potential to be antitumor agents. Compounds **6a**, **6c**, **6g**, **6f** and **6k** showed moderate activity. The best performing three compounds (**6b**, **6h** and **6j**) were evaluated for in silico analysis on the PharmMapper web server, and the human mitogen-activated protein kinase 1 (MEK-1) enzyme was recognized as the main target protein. MEK-1 inhibition by these compounds was further confirmed by the docking study to corroborate the target.

*Graphical Abstract*




**Keywords** Synthesis · Tetrazoles · Antitumor activity · MEK-1 inhibition

## Introduction

Heterocycles play a predominant role in all spheres including pharmaceuticals, natural resources, veterinary, analytical reagents, agriculture products, dyes (Sarvary and Maleki, 2015) and have become the new trend in the development of medicine and pharmaceuticals in modern days (Maddila *et al.*, 2013a; Sarvary and Maleki, 2015). The new approaches for the synthesis of novel heterocycles substituted with unique functional groups form the basis for extensive research activity in synthetic organic chemistry (Roh *et al.*, 2012). Many heterocyclic compounds are in use for industrial, biological and medicinal targets (Myznikov *et al.*, 2007). As resistance to anticancer drugs is snowballing, there is an increasing demand for the novel structure leading that may be of use in designing new potent anticancer agents. Several heterocyclic moieties have been synthesized to develop new molecular entities with promising biological applications.

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The tetrazole derivatives represent an important class of heterocyclic compounds, with nitrogen atoms in their five-membered ring. Several methods have been reported for their synthesis (Habich, 1992; Wittenberger, 1994; Koldobskii *et al.*, 1981; Koldobskii, 2006). The chemistry of tetrazole derivatives has been the subject of intensive research due to their potential biological and pharmacological applications, such as antibacterial (Rostom *et al.*, 2009), antifungal (Upadhayaya *et al.*, 2004), antihistaminic (Giacomo *et al.*, 1999), antiviral (Abdel-Aal *et al.*, 2008), anti-inflammatory (Rajasekaran and Thampi, 2004), antioxidant (Pegklidou *et al.*, 2010), antimalarial (Christophe *et al.*, 2004), anticonvulsant (Abbott and Acheampong, 1988), antipyretic (Luigi *et al.*, 1966), antiallergic (Wittenberger, 1994), antihypertensive and antianxiety agents (Hayao *et al.*, 1967; Sarvary and Maleki 2015). Additionally, they are resistant to metabolic degradation as well as toward chemical oxidants (Yella *et al.*, 2011). Recently, antiproliferative properties of tetrazole derivatives with antitumor applications have also been reported (Gundugola *et al.*, 2010; Romagnoli *et al.*, 2012). Furthermore, *N*-substituted tetrazoles will be advantageous, as these stable compounds can be used for any biological and pharmaceutical trials without the risk of undesirable decomposition (Maddila *et al.*, 2015a). Possibly, the introduction of a substituent group at the *N*-1 position can further increase their antitumor activity.

Cancer is a worldwide health problem and the most frightening disease of humans and animals. In 2008, an estimated 1.38 million cancer-related deaths were reported (GLOBOCAN, 2008). Chemotherapy is often best applied alone or following surgery or radiation of tumors (Nuyttens *et al.*, 2000). Many chemotherapeutic agents usually affect both tumor and normal tissue cells, like bone marrow, intestinal epithelium and hair follicles, alike (Dean *et al.*, 2005). Hence, developing a chemotherapeutic agent with minimal toxicity to the normal cells would be rewarding. Recently, considerable attention has been dedicated to the design of new derivatives of tetrazole moieties due to their potential anticancer activities (Kumar *et al.*, 2011; Kaplancikli *et al.*, 2014). Among the aforementioned compounds, 2-(1-(4-substituted benzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives were promising scaffolds for design of anticancer drugs.

In the recent past, we have reported the synthesis and activity evaluation of various heterocyclic derivatives, such as triazoles (Maddila *et al.*, 2013a) and fused 1,2,4-triazolo-[3,4-*b*][1,3,4]-thiadiazole for anti-inflammatory activity (Maddila *et al.*, 2013b), 1,2,4-triazolo-thieno[2,3-d]pyrimidine for antioxidant activity (Maddila *et al.*, 2013c) and 1,3,4-thiadiazoles for antimicrobial activity (Maddila and Jonnagadda, 2013). In addition, using green and ecofriendly approaches, we have reported the one-pot synthesis of multisubstituted pyridines using Mg–V/$CO_3$ hydrotalcite (Maddila *et al.*, 2015b) or mesoporous $ZrO_2$ (Pagadala *et al.*, 2015a), multi-functionalized benzenes in water using Zn–$VCO_3$ hydrotalcite (Pagadala *et al.*, 2015b), selective oxidation of benzyl alcohol with Mn-based lacunary phosphotungstate supported mesoporous silica as catalyst (Rana *et al.*, 2015) and dihydroquinoline derivatives in aqueous media using ultrasonication (Pagadala *et al.*, 2014). Recently, we have also reported the synthesis of pyrrole-3-carbonitriles with water as solvent and supported by computational validation (Pagadala *et al.*, 2015c). In this work, we focused on the synthesis of novel compounds by encompassing phenylacrylonitrile, substituted benzyl rings conjugated to tetrazole moieties, which could display good antitumor activity against different tumor cell lines.

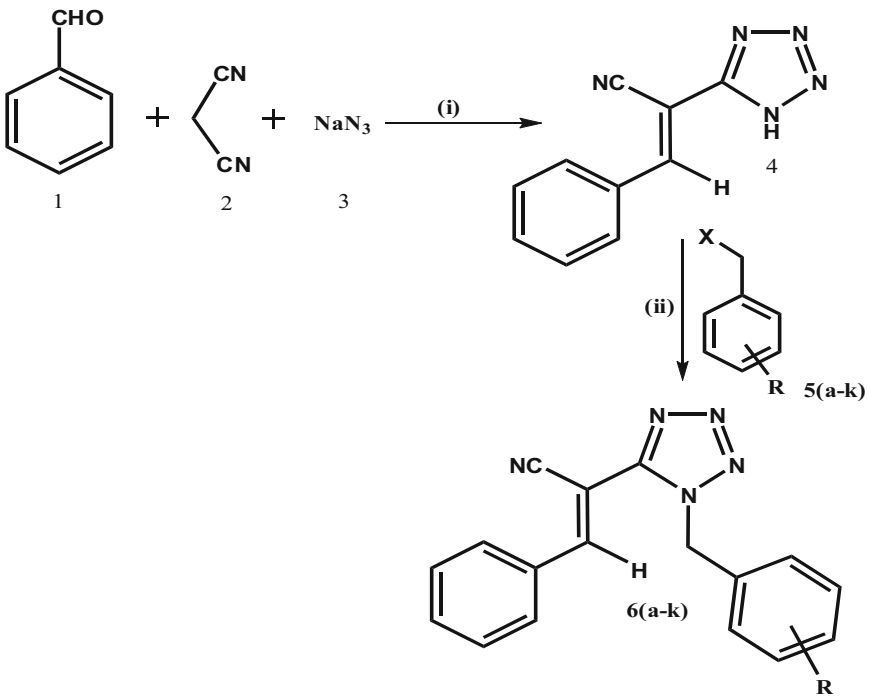
We report a series of novel 2-(1-(4-substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile were synthesized and their structures were confirmed by chemical and spectroscopic methods like FTIR, $^1$H NMR, $^{13}$C NMR spectroscopy and HRMS spectrometry. All the synthesized compounds were screened for their anticancer activity in vitro.

## Results and discussion

### Chemistry

The synthetic pathway for the newly synthesized compounds, 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives (**6a–k**) including the synthesis of intermediary (**4**), is outlined in Scheme 1. The 3-phenyl-2-(1*H*-tetrazol-5-yl)acrylonitrile (**4**), which assisted as an important intermediate, was synthesized starting from aromatic aldehyde, malononitrile and sodium azide in ethanol solvent under reflux condition for 2 h. The reaction of (**4**) with substituted benzyl halides in anhydrous acetone medium afforded 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives (**6a–k**), which on dehydrohalogenation in the presence of $Cs_2CO_3$ catalyst gave the final product. The purity of compounds was confirmed by TLC. All the structures of the novel synthesized compounds were proven by $^1$H NMR, $^{13}$C NMR, HRMS and FTIR spectral analysis. These instrumentation details are given in supporting information (S1).

In infrared spectroscopic analysis of compound **4**, the appearance of bands at 3462, 3017, 2209 and 1566 cm$^{-1}$ correspond to NH, CH–Ar, CN and C=C stretching frequencies respectively. In case of $^1$H NMR spectrum, the broad singlet at 3.69 ppm corresponds to NH proton, sharp singlet at 8.45 ppm to CH proton and 7.12–7.95 ppm for protons in aromatic region. Formation of 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives




**Scheme 1** 2-(1-(Substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile derivatives (**6a–k**). *Reagents and conditions* (*i*) ethanol, reflux, 2 h, (*ii*) $Cs_2CO_3$, dry acetone, reflux, 3 h

| Compound | 6a | 6b | 6c | 6d | 6e | 6f | 6g | 6h | 6i | 6j | 6k |
|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 4-Br | 4-$CF_3$ | 2-Cl | H | 4-OH | 4-MeO | 2-Br | 3,5-F | 2-OH,5-$NO_2$ | 2,4-F | 2,3-MeO |

(**6a–k**) is confirmed by the $^1$H NMR spectrum reflecting a singlet at $\delta$ 4.68–5.27 ppm for $NCH_2$, another singlet at $\delta$ 7.77–8.20 ppm for =CH proton and $\delta$ 6.57–8.02 ppm due to aromatic region protons. The $^{13}$C NMR and HRMS spectral data of compounds **6a–k** are given in "Experimental data" section.

## Anticancer activity

In this study, we evaluated the anticancer activity of new tetrazole derivatives (**6a–k**) against four human cancer cell lines, breast (MCF-7), colon ($CaCO_2$), cervical (HeLa) and (breast) $SkBr_3$, in vitro, by applying the MTT assay. 5-Fluorouracil was used as a positive control, DMSO as a negative control. An observation of the biological activity results detailed in Table 1 indicate that 5-fluorouracil has actively inhibited the human cancer cell lines MCF-7, $CaCO_2$, HeLa and $SkBr_3$ and $IC_{50}$ values of 12, 15, 10 and 26 μM respectively. The most promising compounds, **6b**, **6h** and **6j,** inhibited proliferation of MCF-7, $CaCO_2$, HeLa and $SkBr_3$ cell lines with $IC_{50}$ values of 30, 37, 29 and 35; 51, 49, 38 and 47; and 45, 50, 40 and 49 μM, respectively. In addition, with the exception of the compounds with hydroxy, nitro and simple (**6d**, **6e** and **6i**) substituents, all the other compounds had inactivity against the all MCF-7, CaCO2, HeLa and SkBr3 cell lines.

From preliminary investigations of structure–activity relationships (SARs), the position of the substituent pattern of the *N*-benzyl fragment on the bearing tetrazole backbone was apparently crucial to antitumor activity. In general, compounds with substituents on the phenyl ring showed an enhanced antitumor activity. Compounds with fluoro group on benzyl (**6b**, **6h** and **6j**) exhibited noticeable antitumor potency because of the electron-withdrawing groups (reactive halogens) at the ortho, meta and para position and prove ideal for the cytotoxic activity. Interestingly, the introduction of an additional electron-withdrawing groups such as bromo, chloro (halogens) (**6a**, **6c** and **6g**) and electron donating groups such as methoxy groups (**6f** and **6k**) led to an improvement in antitumor activity than the hydroxyl, nitro and simple substitution (**6d**, **6e** and **6i**). In addition, the position of a halogens and methoxy substituents clearly influenced the cytotoxic action. Introduction of hydroxyl, nitro and simple groups led to declining anti-tumor activity. Notably, the trifluoromethyl-substituted derivative **6b** was very potent against MCF-7, $CaCO_2$, HeLa and $SkBr_3$ cell lines.

## Computational analysis

During the past two decades, drug discovery has mainly concentrated on the single-target paradigm. Now it has

**Table 1** In vitro cytotoxic effect of newly synthesized compounds (**6a–k**)

| Entry | Product | Time (h) | $IC_{50}$ (μM)[a] | | | |
|---|---|---|---|---|---|---|
| | | | MCF-7 | $CaCO_2$ | HeLa | $SkBr_3$ |
| 1 | **6a** | 2.5 | ±88 | ±91 | ±76 | ±81 |
| 2 | **6b** | 3.0 | ±30 | ±37 | ±29 | ±35 |
| 3 | **6c** | 2.5 | ±90 | ±97 | ±69 | ±84 |
| 4 | **6d** | 3.0 | ±115[b] | ±122[b] | ±88 | ±113[b] |
| 5 | **6e** | 3.0 | ±144[b] | ±135[b] | ±74 | ±180[b] |
| 6 | **6f** | 2.5 | ±99 | ±100 | ±81 | ±98 |
| 7 | **6g** | 2.45 | ±98 | ±89 | ±72 | ±87 |
| 8 | **6h** | 3.0 | ±51 | ±49 | ±38 | ±47 |
| 9 | **6i** | 3.0 | ±134[b] | ±119[b] | ±102[b] | ±116[b] |
| 10 | **6j** | 2.3 | ±45 | ±50 | ±40 | ±49 |
| 11 | **6k** | 3.0 | ±87 | ±98 | ±68 | ±91 |
| 12 | 5-Fluorouracil | 0 | ±12 | ±15 | ±10 | ±26 |

[a] $IC_{50}$ value corresponds to the concentration of the respective compound required to affect 50 % mortality in net cells

[b] $IC_{50}$ values >250 μM are considered inactive

shifted toward several target networks by substantial improvement in genomics, and finding the potential receptors for targeted drug has become challenging. In silico methods are generally very effective to examine the potential protein targets and to improve drug discovery of the molecules of interest within short periods and with cost-friendliness. PharmMapper is a web server, which is useful to find the potential target candidates for the given small molecules using pharmacophore mapping method. Of all the newly synthesized compounds in this study, the three best performed compounds (**6b**, **6h** and **6j**) were employed in silico screening for target protein identification on the PharmMapper (Liu *et al.*, 2010). Among the different human proteins investigated, the MEK-1 was found to be potential protein target for **6b**, **6h** and **6j** compounds with their fit scores of 4.43, 4.50 and 4.96, respectively. Figure 1 explains the nine pharmacophore features as *spheres* within the context of the interaction structure in compounds (6b, 6h, 6j). These are as follows: a hydrogen bond acceptor vector, hydrogen bond donor vector, aromatic plane, hydrophobic center, negatively charged center, positively charged center and metal interaction center. The tetrazole ring, benzyl rings, nitrile and fluoro groups were the important structural moieties, which aided interaction with receptor in those compounds.

These observations further substantiate to validate the identified protein compounds that were subsequently docked into the binding site of the protein using the Autodock 4.2 software (Morris *et al.*, 2009). Based on the Autodock score, the conformations were ranked keeping the criteria that lower the binding energy score higher the binding affinity. The binding energies of compounds **6b**

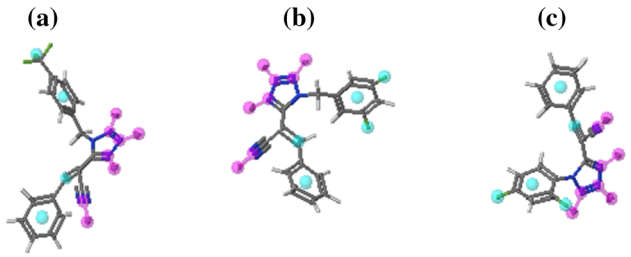



**Fig. 1** Compounds **a 6b**, **b 6h** and **c 6j** showing different pharmacophore features (in *spheres*) identified using the PharmMapper server

(−10.22), **6h** (−10.43) and **6j** (−10.10) show good binding affinities with the targeted protein. The intermol energy values for **6b**, **6h** and **6j** were −11.72, −11.62 and −11.0, respectively.

The docked complexes of the compounds **6b**, **6h** and **6j** with the protein were then visualized in Discovery studio to see a better understanding of the modes of interaction responsible for binding of ligands, which are diagrammatically represented in Fig. 2. The closer assessment of Fig. 2 shows that compound 6b exhibits both hydrogen bonding and hydrophobic interactions to the protein, specifically hydrogen bonds with $PHE^{209}$: N with nitrile nitrogen and halogen fluorine interaction with oxygen of $HIS^{188}$. Moreover, tetrazole ring shows the Pi–sigma interaction with $ILE^{141}$ and Pi–alkyl interaction with $LYS^{97}$. Compound **6h** also interacted with the protein along with hydrophobic interactions with the $VAL^{127}$, $MET^{143}$ and $LEU^{206}$, $ASP^{208}$ amino acids of the protein. Compound **6j** also interacted with protein with the $LEU^{118}$,

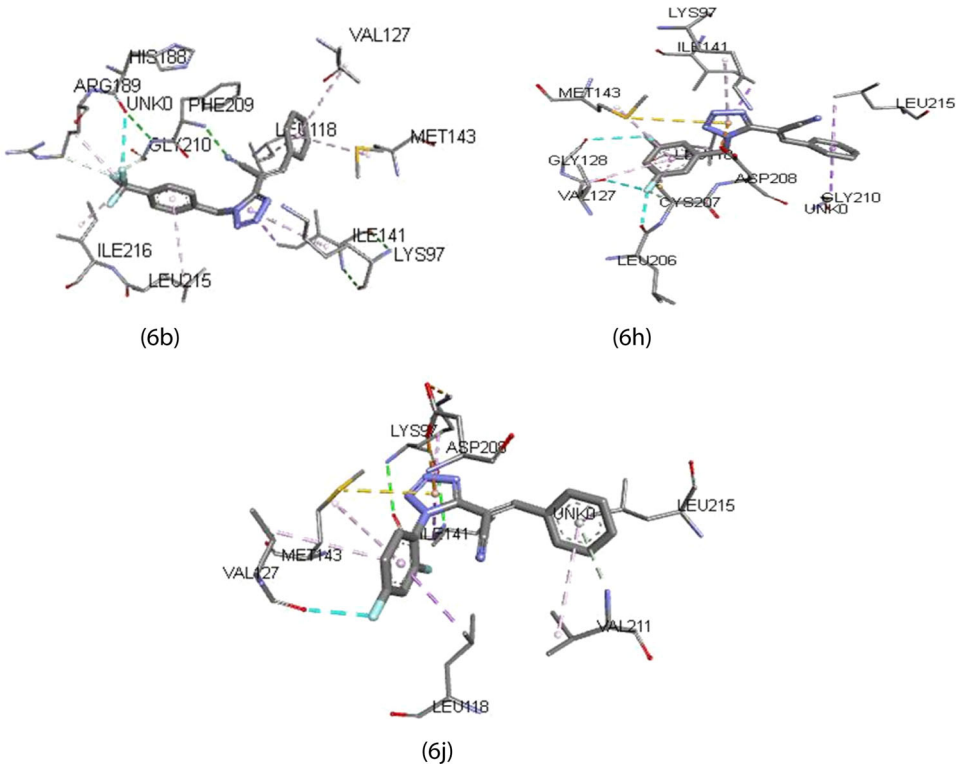



**Fig. 2** Complexes of **6b**, **6h** and **6j** with the MEK-1 protein (pdb code: 1S9J). Only interacting amino acid residues of the protein are shown for clarity purposes. *Orange color* shows the hydrogen bond interaction, and the *light blue color* (Pi–alkyl types) and the *dark blue color* (Pi–sigma types) show the hydrophobic interactions to the ligand (Color figure online)

$VAL^{127}$, $MET^{143}$ and $ASP^{208}$. Method for the computational study is shown in supporting information (S2).

Hence, the active participation of the tetrazole rings, benzyl rings and fluoro substituents of these compounds was found to be very important for locking their geometries in the active site of the MEK-1 receptor.

## Experimental data

### General procedure for the synthesis of 3-phenyl-2-(1*H*-tetrazol-5-yl)acrylonitrile

To a mixture of benzaldehyde **1** (1 mmol), malononitrile (1.1 mmol) and sodium azide (1.2 mmol) in ethanol (10 mL) were added and the reaction mixture was stirred at 70 °C for 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into ice-cold water, and the solid separated was filtered, washed with water, dried and recrystallized from ethanol to obtain compound.

*Synthesis of 3-phenyl-2-(1H-tetrazol-5-yl)acrylonitrile (4)* Light yellow solid, M.P. 170–171 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 3.69$ (br s, 1H, NH), 7.14 (t, $J = 7.20$ Hz, 1H, ArH), 7.22 (d, $J = 7.45$ Hz, 1H, ArH), 7.65 (t, $J = 7.80$ Hz, 2H, ArH), 7.95 (d, $J = 7.45$ Hz, 1H, ArH), 8.45 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 94.8, 114.9, 128.7, 130.1, 132.8, 133.2, 147.9, 156.1; IR (KBr, $cm^{-1}$): 3461, 3017, 2209, 1566, 1255; HRMS of $[C_{10}H_7N5 + H]^+$ (m/z): 197.1109; Calcd.: 197.1105.

### General procedure for the synthesis of 2-(1-(substitutedbenzyl)-1*H*-tetrazol-5-yl)-3-phenylacrylonitrile (6a-k)

A mixture of 3-phenyl-2-(1*H*-tetrazol-5-yl)acrylonitrile (1 mmol), substituted benzyl halides (1 mmol) and cesium carbonate (2 mmol) in dry acetone (10 mL) was refluxed for 3 h. The reaction mixture was monitored by TLC. After completion of the reaction, the solvent was evaporated. The solid was filtered and washed with ice-cold water. Finally, the crude product was purified by recrystallization in ethanol. All the compound details are showed in supplementary information (S3).

*2-(1-(4-Bromobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6a**) White solid, M.P. 168–169 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 5.01$ (s, 2H, $CH_2$), 6.75 (t, $J = 7.30$ Hz, 1H, ArH), 7.06 (d, $J = 8.00$, 2H, ArH), 7.22 (t, $J = 7.80$, 2H, ArH), 7.55 (d, $J = 8.60$, 2H, ArH), 7.59 (d, $J = 8.68$, 2H, ArH), 7.81 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.98, 112.04, 118.96, 120.62, 127.40, 129.10, 131.52, 134.13, 135.11, 145.13; IR (KBr, $cm^{-1}$): 3033, 2225, 1576, 1261, 1069; HRMS of $[C_{17}H_{12}BrN5 + Na]^+$ (m/z): 388.1161; Calcd.: 388.1148.

*2-(1-(4-(Trifluoromethyl)benzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6b**) Yellow solid, M.P. 210–212 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 5.26$ (s, 2H, $CH_2$), 6.78 (t, $J = 7.30$ Hz, 1H, ArH), 7.10 (d, $J = 8.10$, 2H, ArH), 7.24 (t, $J = 7.80$ 2H, ArH), 7.70 (d, $J = 8.10$, 2H, ArH), 7.83 (d, $J = 8.10$ Hz, 2H, ArH), 7.90 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.92, 112.24, 119.36, 122.98, 125.44, 125.48, 125.88, 127.61, 127.61, 129.14, 134.37, 139.89, 144.77; IR (KBr, $cm^{-1}$): 3047, 2220, 1578, 1249, 1021; HRMS of $[C_{18}H_{12}F_3N5 + Na]^+$ (m/z): 378.2042; Calcd.: 378.2036.

*2-(1-(2-Chlorobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6c**) Yellow solid, M.P. 232–233 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 4.75$ (s, 2H, $CH_2$), 6.77 (t, $J = 7.60$ Hz, 1H, ArH), 7.09 (d, $J = 8.30$, 2H, ArH), 7.21–7.33 (m, 3H, ArH), 7.36 (t, $J = 8.10$, 1H, ArH), 7.44 (d, $J = 8.00$ Hz, 1H, ArH), 8.02 (d, $J = 8.80$ Hz, 1H, ArH), 8.20 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.90, 112.16, 119.28, 125.78, 127.33, 129.05, 129.15, 129.67, 131.15, 131.91, 132.90, 144.80; IR (KBr, $cm^{-1}$): 3028, 2220, 1568, 1276, 1019; HRMS of $[C_{17}H_{12}ClN5 + Na]^+$ (m/z): 344.1141; Calcd.: 344.1141.

*2-(1-Benzyl-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6d**) White solid, M.P. 192–194 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 4.68$ (s, 2H, $CH_2$), 6.74 (t, $J = 7.30$ Hz, 1H, ArH), 7.07 (d, $J = 7.95$, 2H, ArH), 7.21 (t, $J = 7.84$, 2H, ArH), 7.28 (t, $J = 7.30$, 1H, ArH), 7.38 (t, $J = 7.70$ Hz, 2H, ArH), 7.65 (d, $J = 8.10$ Hz, 2H, ArH), 7.86 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.90, 111.95, 118.70, 125.58, 127.87, 128.60, 129.07, 135.80, 136.38, 145.26; IR (KBr, $cm^{-1}$): 3116, 2231, 1582, 1281, 1031; HRMS of $[C_{17}H_{13}N5 + H]^+$ (m/z): 288.1040; Calcd.: 288.1039.

*2-(1-(4-Hydroxybenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6e**) Yellow solid, M.P. 185–186 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 4.91$ (s, 2H, $CH_2$), 6.70 (t, $J = 7.28$ Hz, 1H, ArH), 6.78 (d, $J = 8.60$ 2H, ArH), 7.01 (d, $J = 7.70$, 2H, ArH), 7.18 (t, $J = 7.90$, 2H, ArH), 7.46 (d, $J = 8.58$ Hz, 2H, ArH), 7.77 (s, 1H, CH), 10.02 (s, 1H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 50.02, 111.69, 112.05, 115.51, 118.10, 126.87, 127.13, 137.09, 145.66, 157.65; IR (KBr, $cm^{-1}$): 3050, 2224, 1585, 1282, 1038; HRMS of $[C_{17}H_{13}N5O + H]^+$ (m/z): 304.0766; Calcd.: 304.0763.

*2-(1-(4-Methoxybenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6f**) White solid, M.P. 208–209 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 3.77$ (s, 3H, $OCH_3$), 4.95 (s, 2H, $CH_2$), 6.71 (t, $J = 7.24$ Hz, 1H, ArH), 6.95 (d, $J = 8.80$, 2H, ArH), 7.03 (d, $J = 8.10$, 2H, ArH), 7.19 (t, $J = 7.85$, 2H, ArH), 7.57 (d, $J = 8.80$ Hz, 2H, ArH), 7.81 (s, 1H, CH); $^{13}$C NMR (100 MHz, CDCl3): $\delta$ 50.02, 55.14, 111.77, 114.14, 118.29, 126.99, 128.47, 129.02, 133.35, 136.54, 145.54, 159.27; IR (KBr, $cm^{-1}$): 3019, 2224, 1568, 1271, 1072; HRMS of $[C_{18}H_{15}N5O + H]^+$ (m/z): 318.1057; Calcd.: 318.1059.

*2-(1-(2-Bromobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6g**) White solid, M.P. 184–186 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 5.27$ (s, 2H, $CH_2$), 6.77 (t, $J = 7.00$ Hz, 1H, ArH), 7.09 (d, $J = 7.70$, 2H, ArH), 7.21–7.25 (m, 3H, ArH), 7.38 (t, $J = 7.70$ Hz, 1H, ArH), 7.60 (d, 1H, $J = 7.40$ Hz, ArH), 7.99 (d, 1H, $J = 7.40$ Hz, ArH), 8.16 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.98, 112.16, 119.28, 121.65, 126.15, 127.83, 128.80, 129.15, 129.38, 132.90, 134.30, 134.33, 144.80; IR (KBr, $cm^{-1}$): 3033, 2215, 1566, 1255, 1040; HRMS of $[C_{17}H_{12}BrN5 + Na]^+$ (m/z): 388.0124; Calcd.: 388.0113.

*2-(1-(3,5-Difluorobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6h**) Yellow solid, M.P. 221–223 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 5.03$ (s, 2H, $CH_2$), 6.63 (d, 1H, $J = 7.10$ Hz, ArH), 6.72 (t, $J = 7.30$ Hz, 1H, ArH), 6.88 (d, $J = 8.10$ Hz, 2H, ArH), 7.01 (s, 1H, ArH), 7.19–7.28 (m, 3H, ArH), 8.03 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.87, 102.45, 107.43, 111.41, 111.94, 118.48, 129.21, 139.20, 144.97, 156.82, 157.48, 158.76, 158.93, 160.29, 160.33; IR (KBr, $cm^{-1}$): 3011, 2210, 1582, 1224, 1052; HRMS of $[C_{17}H_{11}N5F_2 + H]^+$ (m/z): 324.1397; Calcd.: 324.1396.

*2-(1-(2-Hydroxy-5-nitrobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6i**) Yellow solid, M.P. 189–191 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 4.95$ (s, 2H, $CH_2$), 6.57–6.60 (m, 1H, ArH), 6.69 (d, $J = 8.80$ Hz, 1H, ArH), 6.75 (t, $J = 7.40$ Hz, 1H, ArH), 6.95–6.97 (m, 3H, ArH), 7.22 (t, $J = 7.80$, 2H, ArH), 8.06 (s, 1H, CH), 9.72 (s, 1H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 49.96, 111.65, 112.20, 116.47, 116.54, 118.78, 120.87, 129.21, 136.72, 144.87, 148.43, 149.91; IR (KBr, $cm^{-1}$): 3037, 2223, 1584, 1221, 1057; HRMS of $[C_{17}H_{12}N6O_3 + Na]^+$ (m/z): 371.0389; Calcd.: 371.0395.

*2-(1-(2,4-Difluorobenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6j**) White solid, M.P. 215–216 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 5.07$ (s, 2H, $CH_2$), 6.68–6.74 (m, 1H, ArH), 7.03–7.05 (m, 3H, ArH), 7.10 (s, 1H, ArH), 7.15–7.23 (m, 3H, ArH), 7.77 (s, 1H, CH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 50.02, 111.55, 111.90, 115.28, 117.16, 118.65, 129.08, 129.60, 136.61, 137.08, 145.29, 157.53; IR (KBr, $cm^{-1}$): 3033, 2225, 1580, 1215, 1094; HRMS of $[C_{17}H_{11}N5F_2 + H]^+$ (m/z): 324.0929; Calcd.: 324.0937.

*2-(1-(2,3-Dimethoxybenzyl)-1H-tetrazol-5-yl)-3-phenylacrylonitrile* (**6k**) White solid, M.P. 175–177 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 3.76$ (s, 3H, $OCH_3$), 3.81 (s,

3H, $OCH_3$), 5.26 (s, 2H, $CH_2$), 6.73 (t, $J = 7.30$ Hz, 1H, ArH), 6.96 (d, $J = 8.10$ Hz, 1H, ArH), 7.03–7.06 (m, 3H, ArH), 7.20 (t, $J = 7.80$ Hz, 2H, ArH), 7.47 (d, $J = 7.30$ Hz, 1H, ArH), 8.11 (s, 1H, CH); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 50.22, 55.63, 60.82, 111.89, 112.00, 116.32, 118.71, 124.15, 129.08, 129.19, 131.77, 145.20, 146.33, 152.65; IR (KBr, $cm^{-1}$): 3037, 2230, 1572, 1241, 1060; HRMS of $[C_{19}H_{17}N5O_2 + H]^+$ (m/z): 348.1057; Calcd.: 348.1052.

## In vitro pharmacological studies

### Chemicals

Eagle's minimum essential medium (EMEM) with L-glutamine (4.5 g $L^{-1}$), antibiotics (100×) containing penicillin (10,000 U $mL^{-1}$), streptomycin (10,000 μg $mL^{-1}$) and amphotericin B (25 μg $mL^{-1}$), and trypsin–versene mixture was purchased from Lonza BioWhittaker (Verviers, Liège, Belgium). Gamma-irradiated fetal calf serum (FCS) was purchased from Highveld Biological (Pty) Ltd (Lyndhurst, Gauteng, RSA). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO, cryoprotective medium) and phosphate-buffered saline (PBS) tablets were acquired from Merck (Darmstadt, Hesse, Germany). All tissue culture plastic ware, consumables and cryogenic storage vials were attained from Corning Incorporated (New York City, NY, USA). All other chemicals and reagents were of analytical purity grade or higher and purchased commercially. Ultrapure deionized 18 MΩ water (Milli-Q50) was used throughout.

### Cell lines

MCF-7 (human breast adenocarcinoma), $CaCO_2$ (human epithelial colorectal adenocarcinoma), HeLa (human cervical tumor cells) and SkBr3 (human breast adenocarcinoma) cell lines were routinely propagated in gas-permeable 25-$cm^2$ culture flasks containing 5 mL of EMEM supplemented with 10 % (v/v) gamma-irradiated FCS and antibiotics (100 U $mL^{-1}$ penicillin, 100 μg $mL^{-1}$ streptomycin, 0.25 μg $mL^{-1}$ amphotericin B) at 37 °C under a humidified atmosphere with 5 % $CO_2$ (Thermo Electron Corp. Steri-Cult $CO_2$ incubator, HEPA Class 100). At semi- to full confluence (contact inhibition), cells were split at a 1:3–1:5 ratio every 3–4 days. Cells were freshly cultured and plated 24 h prior to each experiment to maintain the correct pH balance and to eliminate waste product. All preparation of complete culture media, mammalian cell line propagation or maintenance and routine cell culture procedures were performed in a class II Airvolution biological safety cabinet [United Scientific (Pty) Ltd].

### Procedure

Anticancer activity screening of the drugs against four cell lines was quantified in vitro by the MTT reduction assay (van Meerloo *et al.*, 2011), according to reported standard procedure. Briefly, cells were separately trypsinized and seeded into 96-well plates at a density of 2.1–2.5 × $10^4$ cells/well and cultured overnight in 0.1 mL complete medium under the stated conditions to favor cell attachment and to achieve ~80 % cell confluence. Cells were then introduced to different concentrations of the target compounds (25–200 μg $mL^{-1}$; diluent = DMSO @ 1 mg $mL^{-1}$ stock concentrations) and further incubated for 48 h. Following the treatment, cells were then incubated for 4 h with equal volume (0.1 mL) of fresh medium and MTT reagent (5 mg $mL^{-1}$ in sterile PBS, pH 7.4) to allow the formation of formazan by the action of succinate–tetrazolium reductase in living metabolically active cells. Thereafter, cells were rinsed with 0.1 mL of PBS and formazan crystals were solubilized in 0.1 mL DMSO. Absorbance was recorded at 570 nm (detection $\lambda$) and 630 nm (reference $\lambda$) using a Vacutec MR-96A microplate photometer (Vacutec, Hamburg, Germany). Triplicate wells ($n = 3$) were prepared for each individual dose. Control wells containing only cells unexposed to drug were run concurrently. Results were compared to the antiproliferative effects of fluorouracil (5-Fu) as a reference drug. The relationship between surviving ratio and drug concentration was plotted to obtain the survival curve of each cell line as follows: $[(OD_{570}\ \text{Treated} - OD_{630}\ \text{Treated})/(OD_{570}\ \text{Control} - OD_{630}\ \text{Control})] \times 100$ (Sylvester, 2011). $IC_{50}$ response parameter was then deduced which corresponds to the concentration of the individual compound eliciting 50 % mortality in net cells (Ghora *et al.*, 2010).

## Conclusion

In summary, we synthesized a series of phenylacrylonitrile, benzyl substitutes bearing tetrazole derivatives and discovered their antitumor activity. Exclusively, compounds 6b, 6 h and 6j exhibited good anticancer activity and also compounds 6a, 6c, 6g, 6f and 6k showed moderate anticancer activity against four human cancer cell lines, MCF-7, $CaCO_2$, HeLa and $SkBr_3$, in the MTT assay. SAR analysis revealed that trifluoro, difluoro substituents at positions ortho, meta and para on the benzyl ring promoted antitumor activity. Among the tested molecules, compound 6b, 6h and 6j exhibited very good binding affinities and it

shows potential target for anticancer therapy and it can be modified to increase their anticancer activity. In conclusion, the substituent group at the *N*-1 position proved to enhance antitumor activity of the parent compound.

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## References

Abbott FS, Acheampong AA (1988) Quantitative structure-anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. Neuropharmacol 27(3):287–294

Abdel-Aal MT, El-Sayed WA, El-Kosy SM, El-Ashry ESH (2008) Synthesis and antiviral evaluation of novel 5-(n-aryl-aminomethyl-1,3,4-oxadiazol-2-yl) hydrazines and their sugars, 1,2,4-triazoles, tetrazoles and pyrazolyls. Arch Pharm 341(5):307–313

Christophe B, Holger B, Heiner S, Davioud-Charvet E (2004) 5-Substituted tetrazoles as bioisosteres of carboxylic acids. Bioisosterism and mechanistic studies on glutathione reductase inhibitors as antimalarials. J Med Chem 47(24):5972–5983

Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance. Nat Rev Cancer 5:275–284

Ghora MM, Raja FA, Alqasoumi SI, Alafeefy AM, Aboulmagd SA (2010) Synthesis of some new pyrazolo-[3,4-d]-pyrimidine derivatives of expected anticancer and radioprotective activity. Eur J Med Chem 45:171–178

Giacomo BD, Coletta D, Natalini B, Ming-Hong N, Pellicciari R (1999) A new synthesis of carboxyterfenadine (fexofenadine) and its bioisosteric tetrazole analogs. Il Farmaco 54(9):600–610

GLOBOCAN (2008) Lung cancer incidence and mortality worldwide: IARC cancerbase. http://globocan.iarc.fr

Gundugola AS, Chandra KL, Perchellet EM, Waters AM, Perchellet J-PH, Rayat S (2010) Synthesis and antiproliferative evaluation of 5-oxo and 5-thio derivatives of 1,4-diaryl tetrazoles. Bioorg Med Chem Lett 20(3):3920–3924

Habich D (1992) Synthesis of 3′-(5-amino-1,2,3,4-tetrazol-1-yl)-3′-deoxythymidines. Synthesis 4:358–360

Hayao S, Havera HJ, Strycker WG, Leipzig TJ, Rodriguez R (1967) New antihypertensive aminoalkyltetrazoles. J Med Chem 10(3):400–404

Kaplancikli ZA, Yurttas L, Ozdemir A, Turan-Zitouni G, Ciftci GA, Yıldirim SU, Mohsen UA (2014) Synthesis and antiproliferative activity of new 1,5-disubstituted tetrazoles bearing hydrazone moiety. Med Chem Res 23(2):1067–1075

Koldobskii GI (2006) Strategies and prospects in functionalization of tetrazoles. Russ J Org Chem 42:469–486

Koldobskii GI, Ostrovskii VA, Popavskii VS (1981) Advances in the chemistry of tetrazoles. Chem Heterocycl Compd 17:965–988

Kumar CNSSP, Parida DK, Santhoshi A, Kota AK, Sridhar B, Rao VJ (2011) Synthesis and biological evaluation of tetrazole containing compounds as possible anticancer agents. Med Chem Commun 2:486–492

Liu X, Ouyang S, Yu B, Huang K, Liu Y, Gong J, Zheng S, Li Z, Li H, Jiang H (2010) PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. Nucleic Acids Res 38:W609

Luigi A, Luigi P, Alfonso I, Ercolina P, Pierluigi R, Afro G, Amanda O, Walter M (1966) Derivatives of Imidazole. II. Synthesis and reactions of imidazo[1,2-α]pyrimidines and other bi and tricyclic imidazo derivatives with analgesic, antiinflammatory, antipyretic, and anticonvulsant activity. J Med Chem 9(1):29–33

Maddila S, Jonnagadda SB (2013) Synthesis and antimicrobial activity of new 1,3,4-thiadiazoles containing oxadiazole, thiadiazole and triazole nuclei and their antimicrobial activity. Pharm Chem J 46(11):1–6

Maddila S, Pagadala R, Jonnalagadda SB (2013a) 1,2,4-Triazoles: a review of synthetic approaches and the biological activity. Lett Org Chem 10(10):693–714

Maddila S, Gorle S, Singh M, Lavanya P, Jonnalagadda SB (2013b) Synthesis and anti- inflammatory activity of fused 1, 2, 4-triazolo-[3,4-b][1,3,4]thiadiazole derivatives of phenothiazine. Lett Drug Des Discov 10(10):977–983

Maddila S, Avula SK, Gorle S, Moganavelli S, Palakondu L, Jonnalagadda SB (2013c) Synthesis and antioxidant activity of 1,2,4-triazolo linked thieno[2,3-d]pyrimidine derivatives. Lett Drug Des Discov 10(2):186–193

Maddila S, Pagadala R, Jonnalagadda SB (2015a) Synthesis and insecticidal activity of tetrazole linked triazole derivatives. J Heterocycl Chem 52(2):487–499

Maddila S, Rana S, Pagadala R, Jonnalagadda SB (2015b) Res Chem Intermed 41:8269–8278

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 16:2785–2791

Myznikov LV, Hrabalek A, Koldobskii GI (2007) Drugs in the tetrazole series. Chem Heterocycl Compd 43:1–9

Nuyttens JJ, Rust PF, Thomas CR, Turrisi AT (2000) Surgery versus radiation therapy for patients with aggressive fibromatosis or desmoid tumors. Cancer 88(7):1517–1523

Pagadala R, Maddila S, Jonnalagadda SB (2014) Ultrasonic-mediated catalyst-free rapid protocol for the multicomponent synthesis of dihydroquinoline derivatives in aqueous media. Green Chem Lett Rev 7(2):131–136

Pagadala R, Maddila S, Rana S, Kommidi DR, Moodley B, Koorbanally NA, Jonnalagadda SB (2015a) Multicomponent synthesis of pyridines via diamine functionalized mesoporous $ZrO_2$ domino intramolecular tandem Michael type addition. RSC Adv 5:5627–5632

Pagadala R, Maddila S, Rana S, Jonnalagadda SB (2015b) Multicomponent reactions in water medium catalyzed by Zn–$VCO_3$ hydrotalcite: a greener and efficient approach for the synthesis of multifunctionalized benzenes. Curr Org Syn 12(2):163–167

Pagadala R, Kommidi DR, Kankala S, Maddila S, Singh P, Moodley B, Koorbanally NA, Jonnalagadda SB (2015c) Multicomponent one-pot synthesis of highly functionalized pyrrole-3-carbonitriles in aqueous medium and computational study. Org Biomol Chem 1800–1806

Pegklidou K, Koukoulitsa C, Nicolaou I, Demopoulos VJ (2010) Design and synthesis of novel series of pyrrole based chemotypes and their evaluation as selective aldose reductase inhibitors. A case of bioisosterism between a carboxylic acid moiety and that of a tetrazole. Bioorg Med Chem 18(6):2107–2114

Rajasekaran A, Thampi PP (2004) Synthesis and analgesic evaluation of some 5-[β-(10-phenothiazinyl)ethyl]-1-(acyl)-1, 2, 3, 4-tetrazoles. Eur J Med Chem 39(3):273–279

Rana S, Maddila S, Pagadala R, Parida KM, Jonnalagadda SB (2015) Cesium salts of manganese based lacunary phosphotungstate supported mesoporous silica: an efficient catalyst for solvent free oxidation reaction. Catal Commun 59:73–77

Roh J, Vavrova K, Hrabalek A (2012) Synthesis and functionalization of 5-substituted tetrazoles. Eur J Org Chem 27:6101–6118

Romagnoli R, Baraldi PG, Salvador MK, Preti D, Tabrizi MA, Brancale A, Xian-Hua F, Li J, Su-Zhan Z, Hamel E, Bortolozzi R, Basso G, Viola G (2012) Synthesis and evaluation of 1,5-disubstituted tetrazoles as rigid analogues of combretastatin a-4

with potent antiproliferative and antitumor activity. J Med Chem 55(1):475–488

Rostom SAF, Ashour HMA, El Razik HAA, El Fattah AFHA, El-Din NN (2009) Azole antimicrobial pharmacophore-based tetrazoles: synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents. Bioorg Med Chem 17(6):2410–2422

Sarvary A, Maleki A (2015) A review of syntheses of 1,5-disubstituted tetrazole derivatives. Mol Divers 19:189–212

Sylvester PW (2011) Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Des Discov Method Mol Biol 716:157–168

Upadhayaya RS, Jain S, Sinha N, Kishore N, Chandra R, Aror SK (2004) Synthesis of novel substituted tetrazoles having antifungal activity. Eur J Med Chem 39(7):579–592

van Meerloo J, Kaspers GJL, Cloos J (2011) When you can't trust the DNA: RNA editing changes transcript sequences. Method Mol Biol 731:237–245

Wittenberger SJ (1994) Recent developments in tetrazole chemistry. Org Prep Proced Int 26:499–531

Yella R, Khatun N, Rout SK, Patel BK (2011) Tandem regioselective synthesis of tetrazoles and related heterocycles using iodine. Org Biomol Chem 9:3235–3245