ORIGINAL SCIENTIFIC PAPER

*Croat. Chem. Acta* 2021, *94*(4), 191–199
Published online: August 11, 2022
DOI: 10.5562/cca3859

# A Novel Class of Pyrazoline Analogue of Combretastatin-A4 (CA-4): Synthesis Characterization and *in-vitro* Biological Testing

Sadanand N. Shringare,[1,*] Pravin S. Bhale,[2] Hemant V. Chavan,[3] Purva L. Hundekari,[1] Makarand A. Kulkarni[1]

[1] Medicinal Chemistry Research Laboratory, School of Chemical Sciences, Punyashlok Ahilyadevi Holkar Solapur University, Solapur, Maharashtra, 413255, India
[2] Department of Chemistry, Yeshwantrao Chavan Mahavidyalaya, Tuljapur-413 601, Maharashtra, India
[3] Department of Chemistry, A.S.P. College Devrukh (Autonomous), Dist-Ratnagiri-415804, Maharashtra, India
* Corresponding author's e-mail address: snshringare@sus.ac.in

RECEIVED: December 11, 2021 ✶ REVISED: April 22, 2022 ✶ ACCEPTED: April 22, 2022

**Abstract:** A series of pyrazoline bridged combretastatin analogues were designed and synthesised from their precursor chalcone analogues, and all these compounds were ascertained by IR, $^1$H NMR, and mass spectral analysis. Subsequently, all these compounds were evaluated for anticancer activities against breast cancer (MCF-7) and normal Vero (Monkey Kidney) cell lines, and five selected compounds from the series were evaluated against Hela (Human Cervical), MDA-MB-231 (Breast), and A-549 (Lung cancer) cell lines using the Sulforhodamine B (SRB) assay method. Compounds **3a**, **6a**, **6e**, **5b**, **7a**, **5a**, and **7d** were found to be the most potent in the series, with a $GI_{50}$ value of 10 to 30 M in the MCF-7 cell line. Moreover, the same compounds **6a** and **7a** showed remarkable cytotoxicity against the A-549 (Lung) cell line with a $GI_{50}$ value ranging from 10 to 30 M, while compound **3a** displayed moderate cytotoxicity against the Hela (Human Cervical) cell line. All these compounds were found nontoxic to the Vero (Monkey Kidney) normal cell line.

**Keywords**: anticancer, cytotoxicity, pyrazoline, combretastatin.

## INTRODUCTION

CANCER is considered the most serious health issue all over the world. Since most of the currently available chemotherapeutic agents come with multi-drug resistance and acute toxicity.[1] To encounter the future threats arising due to cancer, progressive studies are being conducted towards understanding the biological processes involved in the development of cancer, which may aid in developing more potent and effective anticancer agents for the complete extinction of the disease.[2] Thus, in the pursuit of a more effective anticancer drug, a large number of structurally diverse synthetic and natural products have been explored for their anticancer potential.[3]

Microtubules have been identified as an important structural component of the cytoskeleton and are involved in a wide range of critical cellular functions during cell division.[4] Microtubules are composed of α- and β- tubulin heterodimers that form a dynamic equilibrium in which the tubulin dimers polymerize into long cylindrical microtubules that then depolymerize into individual tubulin units. Therefore, microtubule dynamics is one of the most attractive strategies for the development of cancer therapeutics.[5,6] Any disruption in the dynamic equilibrium blocks cell division and may lead to the induction of mitochondrial apoptosis.[7,8] Small molecules that can disturb microtubule dynamics and cause mitotic arrest are extremely important in current cancer chemotherapy.[9]

Over the past few decades, combretastatin-A4 (CA-4, **1**, Figure 1) has been renowned for being simpler, small molecules, easy to synthesize, and displaying potency by binding with the colchicine binding site and disrupting microtubule polymerization, which induces rapid vascular disruption that eventually leads to tumour cell death.[10] Subsequently, a number of CA-4 analogues have been synthesised in the laboratory that showed substantial anti-tubulin activities. But, unfortunately, most of them failed to

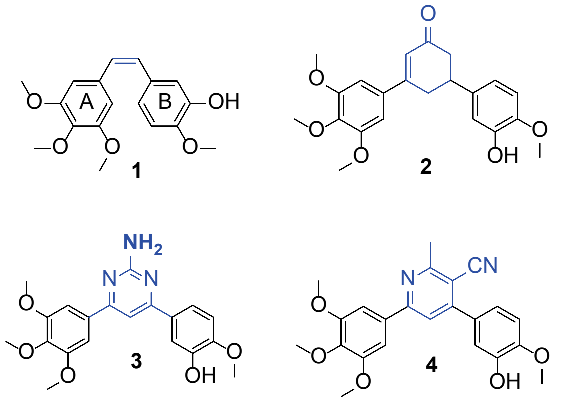



Figure 1. Structures of combretastatin A4 (CA-4, 1), a cyclohexanone (2), a pyrimidine (3), and a pyridine analogue (4).

receive appreciable results clinically due to poor bioavailability.[11–13] Consequently, it is now a challenge to synthesize CA-4 analogues with enhanced clinical potency.

Most combretastatin analogues that have been synthesised thus far have been modified at ring B or at the olefinic bridge.[14] Analysis of these analogues shed light on the fact that ring A with 3,4,5-trimethoxyphenyl groups displays a significant role in disrupting microtubule dynamics. While the cis-stilbene configuration at the bridge in the case of CA-4 has been reported to be essential for potent anti-tubulin polymerization.[15] However, the cis configuration of CA-4 is susceptible to isomerization during storage and after administration, which consequently results in a decrease in the anti-tubulin activity.[16] On the contrary, the CA-4 analogues have been synthesised with replacement of the olefinic bond either with a five-membered or six-membered heterocyclic ring, which not only preserved the appropriate geometric orientation required between the two phenyl rings of CA-4 but is also effective for efficient interaction with the colchicine site of tubulin.[17] For instance, cyclohexanone, pyrazoline, thiophene,[13,18] pyrimidine,[19,20] pyridine,[21,22] (**2**, **3**, and **4** Figure 1). Several of these compounds were synthesised in the author's laboratory and exhibited potent cytotoxic activity.

In continuation of our earlier work to identify possible anticancer agents[19,22–23], we herein aim to report combretastatin CA-4 analogues having the same substituents on the A-ring while altering the B-ring and stilbene bridgehead linker with pyrazoline, a 3-carbon linker, as possible anticancer agents.

# EXPERIMENTAL SECTION

## Materials and Methods

All Chemicals required for the experiment were of AR/GR quality and purchased from Aldrich (Sigma–Aldrich), Spectrochem, or Lancaster (Alfa Aesar, Johnson Matthey Company) and used without any purification. The reaction course was monitored by TLC using chloroform/methanol as the mobile phase on pre-coated $F_{254}$ Merck TLC plates and visualized in a UV/fluorescent analysis cabinet and/or iodine chamber. Organic solutions obtained after the reaction were dried over anhydrous $Na_2SO_4$. The melting points of compounds were determined with a digital thermometer and are uncorrected. Infrared (IR) spectra of compounds were recorded on the Infrared FT-IR Spectrometer, Nicolet iS10; Thermo Electron Scientific, USA and values were denoted in $cm^{-1}$. The mass spectra of compounds were established on the Shimadzu LCMS-2010 EV (Maharashtra, India). $^1H$ NMR and $^{13}C$ NMR spectra were recorded at 400 MHz on the FT-Nuclear Magnetic Resonance Spectrometer (FT-NMR), Bruker AVIII, Switzerland using $CDCl_3$ solvent and chemical shift valves were noted in parts per million on $\delta$ scale. The coupling constant (**J**) is denoted in hertz (Hz). All the compounds showed $^1H$ NMR spectra in agreement with the assigned structures.

# SYNTHESIS

General method for the synthesis of chalcone and pyrazoline analogue of combretastatin-A4 (CA-4) derivatives **3a**, **4a**, **5a**–**c**, **6a**–**e**, and **7a**–**e**

### (E)-1-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3a)

To a suspension of 1-(4-nitrophenyl) ethanone **1** (1.65 g, 10 mmol) in 15 mL ethanol, 3,4,5-trimethoxybenzaldehyde **2** (1.96 g, 10 mmol) was added. When all content becomes soluble, 10 % (5 mL) sodium hydroxide was added slowly while maintaining ice-cold condition and the mixture was stirred at room temperature. Progress of the reaction was monitored by TLC. After completion of the reaction (24 h), the mixture was quenched in ice-cold water. The precipitate obtained was stirred, filtered, washed with water, and finally purified by crystallization in ethanol to obtain yellow compound **3a**.

Yield: 274 mg, 80 %. MP: 152–154 °C; Molecular Formula $C_{18}H_{17}NO_6$/343.11; IR (KBr, $cm^{-1}$): 2940 (C=C–H), 2838 (C–H), 1610 (C=O), 1545 (C=C), 1328 $NO_2$ sym), 1519 ($NO_2$ asym); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 3.938 (s, 6H, $-OCH_3$); 3.951 (s, 3H, $-OCH_3$); 6.896 (s, 2H, ArH); 7.372 (d, 1H, $J$ = 15.6 Hz, C=C–H); 7.772 (d, 1H, $J$ = 15.6 Hz, C=C–H ); 8.158 (d, 2H, $J$ = 8.8Hz, ArH); 8.379 (d, 2H, $J$ = 8.8Hz, ArH); MS: $m/z$ 344 (M+H)

### (3-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole (4a)

To a suspension of (E)-1-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **3a** (0343 g, 1.0 mmol) in 5 mL absolute ethanol, hydrazine hydrate (0.2 g, 4.0 mmol) was added dropwise and the mixture was stirred under reflux. Progress of the reaction was monitored by TLC. After completion of the reaction (6 h), the mixture was allowed to cool at room temperature. The precipitate obtained was filtered, washed with hot ethanol (5 × 3 mL), and dried under vacuum to obtain yellow product **4a**.

Yield: 285 mg, 80 %; M.F./MW.: $C_{18}H_{19}N_3O_5$/357.36; M.P.: 162–164 °C; IR (cm$^{-1}$): 3302 (–NH), 1591 (C=N), 1555 (C=C), 1327 ($NO_2$ sym), 1505 ($NO_2$ asym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.058 (dd, 1H, $J$ = 10 Hz, $J$ = 16 Hz, –$CH_2$–pyrazoline); 3.520 (dd, 1H, $J$ = 11.2 Hz, $J$ = 16.4 Hz, –$CH_2$–pyrazoline); 3.864 (s, 3H, –$OCH_3$); 3.879 (s, 6H, 2×–$OCH_3$); 4.998 (dd, 1H, $J$ = 10.4 Hz, $J$ = 19.6 Hz, –CH–pyrazoline); 6.305 (s, 1H, –NH); 6.629 (s, 2H, ArH); 7.802 (d, 2H, $J$ = 9.2 Hz, ArH); 8.252 (d, 2H, $J$ = 9.2 Hz, ArH); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 41.09, 56.16, 61.0, 65.35, 103.12, 124, 126.20, 135.23, 137.47, 137.55, 139, 147.34, 148.44, 153.58; HRMS: calculated: *m/z* 357.1325, found: *m/z* 358.1406 (M+H).

### 5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde (5a)

To a suspension of (E)-1-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **3a** (343 mg, 1.0 mmol) in 10 mL formic acid, hydrazine hydrate (0.2 g, 4.0 mmol) was added dropwise and the mixture was stirred under reflux. The progress of the reaction was monitored by TLC. After completion of the reaction (5 h), the mixture was poured over ice-cold water, the precipitate so formed was filtered, washed with water, air-dried, and finally purified by column chromatography using hexane: ethyl acetate (7:3) to obtain pale yellow solid.

Yield: 277 mg, 72 % M.F./MW.: $C_{19}H_{19}N_3O_6$ / 385; M.P.: 80–82 °C IR (cm$^{-1}$): 2839 (C=C–H), 1670 (C=O), 1594 ($NO_2$ asym) and 1345 ($NO_2$ sym); 1519 (C=C), 1281 and 1123 (C–O); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 3.249 (dd, 1H, $J$ = 5.2 Hz, $J$ = 17.6 Hz, –$CH_2$–pyrazoline); 3.784 (s, 6H, –$OCH_3$); 3.813 (s, 3H, –$OCH_3$); 3.946–3.820 (m, 1H, –$CH_2$–pyrazoline ); 5.54 (dd, 1H, $J$ = 5.2 Hz, $J$ = 11.6 Hz, –CH–pyrazoline); 6.428 (s, 2H, ArH); 7.908 (d, 2H, $J$ = 8.8 Hz, ArH); 8.298 (d, 2H, $J$ = 8.8 Hz, ArH); 9.037 ( s, 1H, ArCHO); MS: m/386 (M+H).

### 1-[5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-ethanone (5b)

To a suspension of (E)-1-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **3a** (343 mg, 1.0 mmol) in 10 mL acetic acid, hydrazine hydrate (0.2 g, 4.0 mmol) was added dropwise and the mixture was stirred under reflux. The progress of the reaction was monitored by TLC. After completion of the reaction (5 h), the mixture was poured over ice-cold water, and the precipitated solid was filtered, washed with water, air-dried, and finally purified by column chromatography using hexane: ethyl acetate (7:3) to obtain a pale-yellow product **5b**.

Yield: 307 mg, 77 % M.F./MW.: $C_{20}H_{21}N_3O_6$ / 399; M.P.: 140–142 °C IR (cm$^{-1}$): 2837 (C=C–H), 1671 (C=O), 1573 ($NO_2$ asym) and 1340 ($NO_2$ sym); 1512 (C=C), 1248 and 1122 (C–O); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 2.474 (s, 3H, $CH_3$); 3.194 (dd, 1H, $J$ = 5.2 Hz, $J$ = 18.0 Hz, –$CH_2$–pyrazoline); 3.799 (s, 6H, –$OCH_3$); 3.819 (s, 3H, –$OCH_3$); 3.936–3.840 (m, 1H, –$CH_2$–pyrazoline ); 5.578 (dd, 1H, $J$ = 4.8 Hz, $J$ = 12.0 Hz, –CH–pyrazoline); 6.404 (s, 2H, ArH); 7.899 (d, 2H, $J$ = 8.8 Hz, ArH); 8.28 (d, 2H, $J$ = 8.8 Hz, ArH); HRMS: calculated: *m/z* 399.1430, found: *m/z* 400.1503 (M+H).

### 1-[5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-propan-1-one (5c)

To a suspension of (E)-1-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **3a** (343 mg, 1.0 mmol) in 10 mL propionic acid, hydrazine hydrate (0.2 g, 4.0 mmol) was added dropwise and the mixture was stirred under reflux. Progress of the reaction was monitored by TLC. After completion of the reaction (5 h), the mixture was poured over ice-cold water, the precipitate obtained was filtered, washed with water, air-dried, and finally purified by column chromatography using hexane: ethyl acetate (7:3) to obtain pale yellow compound **5c**.

Yield: 326 mg, 79 %; M.F./MW.: $C_{21}H_{23}N_3O_6$ / 413; M.P.: 161–163 °C; IR (cm$^{-1}$): 2838 (C=C–H), 1672 (C=O), 1594 ($NO_2$ asym) and 1343 ($NO_2$ sym); 1509 (C=C), 1237 and 1125 (C–O); $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.4 (t, 2H, $J$ = 7.6 Hz); 2.854 (q, 3H, $J$ = 15.6 Hz, –$CH_3$); 3.180 (dd, 1H, $J$ = 5.2 Hz, $J$ = 17.6 Hz, –$CH_2$–pyrazoline); 3.718 (s, 3H, –$OCH_3$); 3.763 (s, 6H, –$OCH_3$); 3.952–3.777 (m, 1H, –$CH_2$–pyrazoline); 5.574 (dd, 1H, $J$ = 5.2 Hz, $J$ = 12.0 Hz, –CH–pyrazoline); 6.404 (s, 2H, ArH); 7.902 (d, 2H, $J$ = 7.2 Hz, ArH); 8.292 (d, 2H, $J$ = 7.2 Hz, ArH); MS: *m/z* 414 (M+H).

### General procedure for the preparation of N$^1$-phenyl pyrazoline analogues of CA-4 (6a–e)

To a suspension of **3a** (1 mmol) in 5 mL absolute ethanol, substituted phenylhydrazine (1.0 mmol) was added and the mixture was stirred at reflux for 6 h. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. Upon cooling, the solid precipitated out, which was filtered, washed with hot ethanol (2 × 3mL), and dried under vacuum to obtain the title compounds (**6a**–**e**).

### 5-(4-Nitro-phenyl)-1-o-tolyl-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (6a)

Yield: 299 mg, 67 %; M.F./MW.: $C_{25}H_{25}N_3O_5$/ 447.48; M.P: 131–133 °C; IR (cm$^{-1}$): 2922 (C=C–H), 2839 (C–H), 1581 (C=N), 1558 (C=C), 1519 ($NO_2$ asym), 1310 ($NO_2$ sym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.026 (s, 3H); 3.222 (dd, 1H, $J$ = 10.4 Hz, $J$ = 10.8 Hz, –$CH_2$–pyrazoline); 3.635 (s, 6H, $OCH_3$); 3.836 (s, 3H, $OCH_3$); 3.974–3.8886 (m, 1H, –$CH_2$–pyrazoline); 5.393 (dd,1H, $J$ = 11.2 Hz, $J$ = 11.2 Hz, –CH–pyrazoline);

6.414 (s, 2H, ArH); 7.400–7.265 (m, 4H, ArH); 8.083 (d, 2H, *J* = 8.8 Hz, ArH), 8.293 (d, 2H, *J* = 8.4 Hz, ArH): MS: *m/z* 448 (M+H).

**5-(4-Nitro-phenyl)-1-p-tolyl-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (6b)**

Yield: 326 mg, 73 %; M.F./MW.: $C_{25}H_{25}N_3O_5$/ 447.48; M.P: 150–152 °C; IR (cm$^{-1}$): 2941 (C=C–H), 2834 (C–H), 1577 (C=N), 1563 (C=C), 1523 ($NO_2$ asym), 1317 ($NO_2$ sym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.276 (s, 3H); 3.168 (dd, 1H, *J* = 7.6 Hz, *J* =16.8 Hz, –$CH_2$–pyrazoline ); 3.809 (s, 3H, $OCH_3$); 3.840 (s, 6H, $OCH_3$); 3.942–3.877 (m, 1H, –$CH_2$–pyrazoline); 5.269 (dd, 1H, *J* = 8.0 Hz, *J* = 12.8 Hz, –CH–pyrazoline); 6.510 (s, 2H, ArH); 7.040 (s, 4H, ArH); 7.820 (d, 2H, *J* = 9.2 Hz, ArH), 8.24 (d, 2H, *J* = 8.8 Hz, ArH); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 20.57, 43, 56.17, 61, 65.71, 102.35, 114, 124.01, 125.73, 129.59, 129.91, 137.33, 137.67, 139.01, 141.77, 143.70, 146.91, 153.93; MS: *m/z* 448 (M+H).

**1-(4–CHloro-phenyl)-3-(4-nitro-phenyl)-5-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (6c)**

Yield: 359 mg, 77 %; M.F./MW.: $C_{24}H_{22}N_3O_5Cl$/ 467.90; M.P: 142–144 °C; IR (cm$^{-1}$): 2928 (C=C–H), 2830 (C–H), 1590 (C=N), 1550 (C=C), 1508 ($NO_2$ asym), 1323 ($NO_2$ sym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.201 (dd, 1H, *J* = 7.6 Hz, *J* = 17.2 Hz, –$CH_2$–pyrazoline ); 3.814 (s, 3H, $OCH_3$); 3.878 (s, 6H, $OCH_3$); 3.910–3.867 (m, 1H, –$CH_2$–pyrazoline); 5.275 (dd, 1H, *J* = 7.6 Hz, *J* = 12.8 Hz, –CH–pyrazoline); 6.474 (s, 2H, ArH); 7.047 (d, 2H, *J* = 6.8Hz, ArH); 7.184 (d, 2H, *J* = 6.8 Hz, ArH), 7.834 (d, 2H, *J* = 7.2 Hz, ArH), 8.254 (d, 2H, *J* = 7.2 Hz, ArH); $^{13}$C NMR(400 MHz, $CDCl_3$): $\delta$ = 43.12, 56.20, 60.85, 65.39, 102.25, 114.98, 124.03, 125.32, 125.98, 128.99, 136.92, 137.54, 138.56, 142.44, 144.87, 147.23, 154.05; HRMS: calculated: *m/z* 467.1248, found: *m/z* 468.290 (M+H).

**4-[5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzonitrile (6d)**

Yield: 329 mg, 72 %; M.F./MW.: $C_{25}H_{22}N_4O_5$/ 458.57; M.P: 182–184 °C; IR (cm$^{-1}$): 2938 (C=C–H), 2828 (C–H), 2224 (–CN), 1603 (C=N), 1547 (C=C), 1505 ($NO_2$ asym), 1312 ($NO_2$ sym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.266 (dd, 1H, *J* = 6.8 Hz, *J* = 17.6 Hz, –$CH_2$–pyrazoline ); 3.808 (s, 3H, $OCH_3$); 3.836 (s, 6H, $OCH_3$); 3.931 (dd, 1H, *J* = 12.4 Hz, *J* = 17.2 Hz, –$CH_2$–pyrazoline); 5.363 (dd, 1H, *J* = 6.4 Hz, *J* = 12.4 Hz, –CH–pyrazoline); 6.434 (s, 2H, ArH); 7.137 (d, 2H, *J* = 8.8 Hz, ArH); 7.495 (d, 2H, *J* = 8.8 Hz, ArH), 7.876 (d, 2H, *J* = 9.2 Hz, ArH), 8.276 (d, 2H, *J* = 8.8 Hz, ArH); MS: *m/z* 459 (M+H).

**1-(4-Fluoro-phenyl)-5-(4-nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (6e)**

Yield: 333 mg, 74 %; M.F./MW.: $C_{24}H_{22}N_3O_5F$/ 451.45; M.P: 174–176 °C; IR (cm$^{-1}$): 2970 (C=C–H), 2930 (C–H), 1593 (C=N), 1548 (C=C), 1502 ($NO_2$ asym), 1331 ($NO_2$ sym); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.192 (dd, 1H, *J* = 8.0 Hz, *J* = 17.2 Hz, –$CH_2$–pyrazoline); 3.820 (s, 3H, $OCH_3$); 3.845 (s, 6H, $OCH_3$); 3.901–3.858 (m, 1H, –$CH_2$–pyrazoline); 5.248 (dd, 1H, *J* = 8.0 Hz, *J* = 12.8 Hz, –CH–pyrazoline); 6.502 (s, 2H, ArH); 6.944 (d, 2H, J = 8.4 Hz, ArH); 7.073 (d, 2H, *J* = 9.2 Hz, ArH), 7.826 (d, 2H, *J* = 6.8 Hz, ArH), 8.251 (d, 2H, *J* = 6.8 Hz, ArH); $^{13}$C NMR(400 MHz, $CDCl_3$): $\delta$ = 43.18, 56.20, 60.85, 66.01, 102.41, 115.08, 115.54, 115.77 124.03, 125.86, 137.17, 138.74, 140.53, 144.40, 147.14, 154.03, 158.75; MS: *m/z* 452 (M+H)

**General procedure for the preparation of 3, 5-diaryl-1-carbothioamide–pyrazoline (7a–e)**

To a suspension of (3-(4-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole **4a** (1 mmol) in 5 mL absolute ethanol, substituted phenyl isothiocyanate (1.0 mmol) was added and the mixture was stirred under reflux for 1 h. After completion of the reaction (monitored by TLC), the mixture was allowed to cool to room temperature. Upon cooling, solid precipitate out, which was filtered, washed with hot ethanol (2 × 3mL), and dried under vacuum to obtain the title compound (**7a**–**e**).

**5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid o-tolylamide (7a)**

Yield: 364 mg, 72 %; M.F./MW.: $C_{26}H_{26}N_4O_5S$/ 506.57; M.P: 202–204 °C; IR (cm$^{-1}$): 3322 (NH), 2923(C=C–H), 1577 (C=N), 1517 ($NO_2$ asym), 1336 ($NO_2$ sym), 1501 (C=C), 1302 (C=S); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.374 (s, 3H ); 3.287 (dd, 1H, *J* = 3.6 Hz, *J* = 17.6 Hz, –$CH_2$–pyrazoline); 3.835 (s, 3H, $OCH_3$); 3.849 (s, 6H, $OCH_3$); 3.959–3.869 (m, 1H, –$CH_2$–pyrazoline); 6.207 (dd, 1H, *J* = 3.6 Hz, *J* = 11.6 Hz, –CH–pyrazoline); 6.451 (s, 2H, ArH); 7.298–7.236 (m, 3H, ArH); 7.576 (dd, 1H, *J* = 1.6 Hz, J = 8.4 Hz, ArH); 7.93 (d, 2H, *J* = 8.8 Hz, ArH); 8.315 (d, 2H, *J* = 8.8 Hz; ArH); 9.011 (s, 1H, –NH); MS: m/e 507 (M+H).

**5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid p-tolylamide (7b)**

Yield: 394 mg, 78 %; M.F./MW.: $C_{26}H_{26}N_4O_5S$/ 506.57; M.P: 228–230 °C; IR (cm$^{-1}$): 3339 (NH), 2941(C=C–H), 1567 (C=N), 1518 ($NO_2$ asym), 1329 ($NO_2$ sym), 1491 (C=C), 1297 (C=S); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.355 (s, 3H ); 3.27 (dd, 1H, *J* = 4.0 Hz, *J* = 18.0 Hz, –$CH_2$–pyrazoline–H); 3.824 (s, 3H, $OCH_3$); 3.835 (s, 6H, $OCH_3$); 3.929–3.849 (m, 1H, –$CH_2$–pyrazoline); 6.185 (dd, 1H, *J* = 3.6 Hz, *J* = 11.6 Hz, –CH–pyrazoline); 6.386 (s, 2H, ArH); 7.192 (d, 2H, *J* = 8.0 Hz, ArH); 7.478 (d, 2H, *J* = 8.4 Hz, ArH); 7.935 (d, 2H, *J* = 8.8 Hz, ArH); 8.304 (d, 2H, *J* = 8.8 Hz, ArH); 9.197 (s, 1H, –NH); MS: *m/e* 527 (M+H).

**5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid (4–CHloro-phenyl)-amide (7c)**

Yield: 405 mg, 77 %; M.F./MW.: $C_{25}H_{23}ClN_4O_5S$/ 526.99; M.P: 210–212 °C; IR (cm$^{-1}$): 3313 (NH), 2928(C=C–H), 1593 (C=N), 1505 ($NO_2$ asym), 1328 ($NO_2$ sym), 1459 (C=C), 1289

(C=S); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.287 (dd,1H, $J$ = 3.2 Hz, $J$ = 18.0 Hz, –$CH_2$–pyrazoline H); 3.777 (s, 3H, $OCH_3$); 3.834 (s, 6H, $OCH_3$); 3.945–3.900 (m, 1H, –$CH_2$–pyrazoline); 6.152 (dd, 1H, $J$ = 8.4 Hz, $J$ = 11.2 Hz, –CH–pyrazoline); 6.443 (s, 2H, ArH); 7.310 (d, 2H, $J$ = 8.4 Hz, ArH); 7.608 (d, 2H, $J$ = 8.4 Hz, ArH); 7.942 (d, 2H, $J$ = 8.4 Hz, ArH); 8.310 (d, 2H, $J$ = 8.4 Hz, ArH); 9.234 (s, 1H, –NH); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 42.56, 56.21, 60.82, 64.04, 102.33, 124.20, 125.64, 127.63, 128.83, 131.11, 136.51, 136.88, 137.08, 137.52, 148.86, 152.82, 153.75, 174.49; MS: m/e 527 (M+H).

**5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid (4-cyano-phenyl)-amide (7d)**

Yield: 382 mg, 74 %; M.F./MW.: $C_{26}H_{23}N_5O_5S$/ 517.56; M.P: 238–240 °C; IR (cm$^{-1}$): 3303 (NH), 2938 (C=C–H), 2220 (–C≡N), 1591 (C=N), 1514 ($NO_2$ asym), 1344 ($NO_2$ sym), 1494 (C=C), 1305 (C=S); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.322 (dd, 1H, $J$ = 3.6 Hz, $J$ = 18.0 Hz, –$CH_2$–pyrazoline H); 3.827 (s, 3H, $OCH_3$); 3.832 (s, 6H, $OCH_3$); 3.976–3.864 (m, 1H, –$CH_2$–pyrazoline); 6.143 (dd, 1H, $J$ = 4.0 Hz, $J$ = 12.0 Hz, –CH–pyrazoline); 6.435 (s, 2H, ArH); 7.652 (d, 2H, $J$ = 8.4 Hz, ArH); 7.942 (d, 2H, $J$ = 2.8 Hz, ArH); 7.964 (d, 2H, $J$ = 3.2 Hz, ArH); 8.326 (d, 2H, $J$ = 8.8 Hz, ArH); 9.488 (s, 1H, –NH); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 42.67, 56.24, 60.85, 64.02, 102.32, 108.10, 118.77, 122.77, 124.28, 127.75, 132.85, 136.19, 136.68, 137.62, 142.44, 149.02, 153.43, 153.80, 173.16; MS: m/e 527 (M+H).

**5-(4-Nitro-phenyl)-3-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid (2-methoxy-phenyl)-amide (7e)**

Yield: 381 mg, 73 %; M. F. /Mw: $C_{26}H_{26}N_4O_6S$/522.57; M.P: 218–220 °C; IR (cm$^{-1}$): 3349 (NH), 2927 (C=C–H), 1587 (C=N), 1509 ($NO_2$ asym), 1339 ($NO_2$ sym), 1487 (C=C), 1312 (C=S); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 3.27 (dd,1H, $J$ = 3.6 Hz, $J$ = 17.6 Hz, –$CH_2$–pyrazoline); 3.826 (s, 3H, $OCH_3$ ); 3.837 (s, 6H, $OCH_3$); 3.922–3.868 (m, 1H, –$CH_2$–pyrazoline); 3.972 (s, 3H, $OCH_3$); 6.205 (dd, 1H, $J$ = 4.0 Hz, $J$ = 11.6 Hz, –CH–pyrazoline); 6.456 (s, 2H, ArH); 6.984 (dd, 2H, $J$ = 7.6 Hz, J = 17.2Hz, ArH); 7.144 (dd, 1H, $J$ = 1.6 Hz, J = 8.0Hz, ArH); 7.936(d, 2H, $J$ = 8.8 Hz, ArH); 8.325 (d, 2H, $J$ = 8.8 Hz; ArH); 8.69 (dd, 1H, $J$ = 1.6 Hz, $J$ = 8.0Hz, ArH); 9.886 (s, 1H, –NH); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$ = 42.43, 56.05, 56.18, 60.81, 63.90, 102.26, 110.37, 120.46, 122.29, 124.19, 125.12, 127.44, 128.0, 136.94, 137.38, 148.68, 150.11, 151.88, 153.72, 173.35; MS: m/e 523 (M+H).

## Anticancer Activity

### Experimental Procedure for SRB Assay

The cytotoxic activity of the compounds was measured against five human cell lines: MCF-7 (breast carcinoma) Hela (human Cervical), MDA-MB-231 (Breast), A-549 (Lung) and Vero (Monkey kidney normal cell line) (breast) according to the previously published standard SRB assay drug treatment experimental procedure.[24,25]

The cell lines were grown in RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % $CO_2$, 95 % air, and 100 % relative humidity for 24 h prior to the addition of experimental drugs. Experimental drugs were solubilized in an appropriate solvent to prepare a stock of $10^{-2}$ concentrations. At the time of the experiment, four 10-fold serial dilutions were made using the complete medium. Aliquots of 10 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µL of the medium, resulting in the required final drug concentrations.

After compound addition, plates were incubated at standard conditions for 48 hours and the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µL of cold 30 % (*w*/*v*) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4 % (*w*/*v*) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, the unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air-dried. The bound stain was subsequently eluted with a 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with a 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells × 100. Using the six absorbance measurements [time zero ($Tz$), control growth ($C$), and test growth in the presence of drug at the four concentration levels ($Ti$)], the percentage growth was calculated at each of the drug concentrations levels. The dose-response parameters were calculated for each test article. Growth inhibition of 50 % ($GI_{50}$) was calculated from $[(Ti - Tz) / (C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The $LC_{50}$ (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicates a net loss of cells following treatment is calculated from $[(Ti - Tz) / Tz] \times 100 = -50$.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

## RESULT AND DISCUSSION

### Chemistry

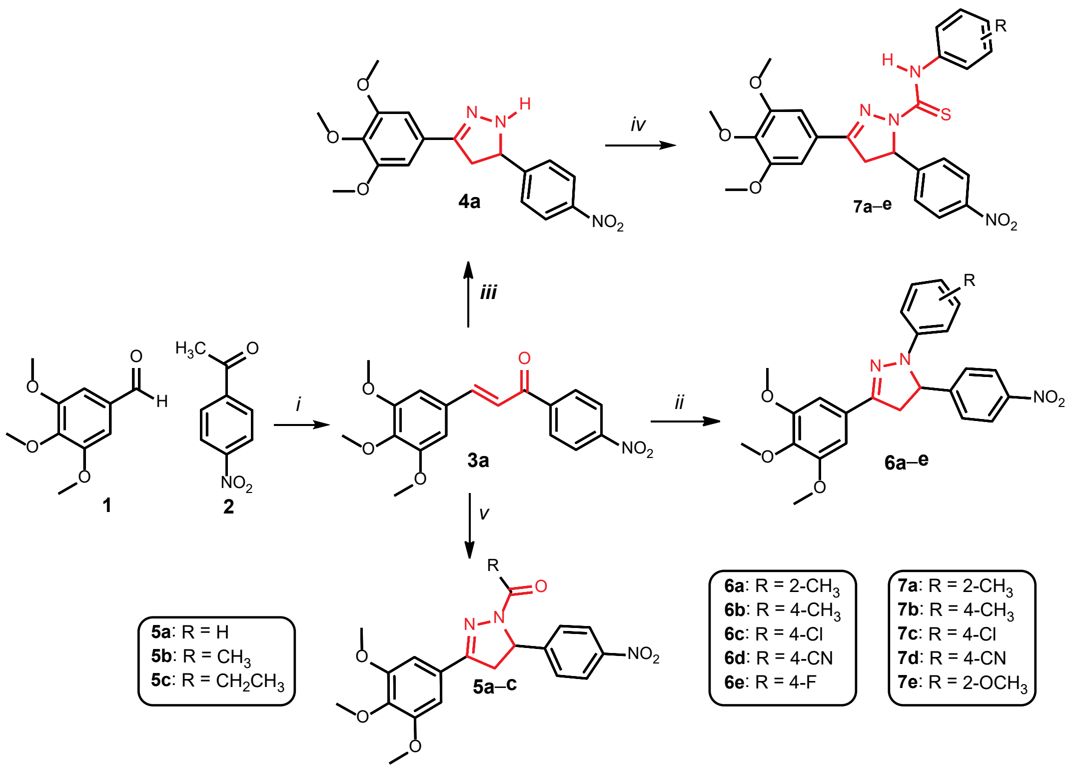
In the current study, we report three categories of novel analogues of combretastatin-A4 with pyrazoline bridgehead linker having the same substituent on the A ring while the B ring is replaced with a 4-nitro-phenyl group, such as, N[1]-acetylated pyrazoline analogue of CA-4 (**5a**–**c**), N[1]-phenylpyrazoline of CA-4 (**6a**–**e**) and 3, 5-diaryl-1-carbothioamide-pyrazoline of CA-4 (**7a**–**e**). The synthesis of target compounds (**5a**–**c**) was achieved by treating precursor chalcone **3a** separately with HCOOH, $CH_3COOH$, and $CH_3CH_2COOH$ in presence of hydrazine hydrate under reflux for 6 h (Scheme 1). Further, compounds (**6a**–**e**) were obtained by reacting **3a** with differently substituted phenylhydrazine hydrochlorides in ethanol under the catalyst-free condition in good yield (Scheme 1). On the other hand, the synthesis of compounds (**7a**–**e**) was achieved by treating pyrazoline analogue **4a** with differently substituted phenyl isothiocyanates. However, the pyrazoline analogue of CA-4 (**4a**) was synthesized by refluxing precursor chalcone **3a** with hydrazine hydrate in absolute ethanol (Scheme 1).

The structures of all the newly synthesised- N[1]-acetylated pyrazoline analogues of CA-4 (**5a**–**c**), N[1]-phenyl pyrazolines (**6a**–**e**) and 3, 5-diaryl-1-carbothioamide-pyrazoline (**7a**–**e**) derivatives were confirmed by $^{1}$H NMR, $^{13}$C NMR, IR and mass spectrometry. All the synthesised pyrazoline analogues show three characteristics *doublet of doublet* (*dd*) peaks in $^{1}$H NMR spectrum for –CH proton present adjacent to $–CH_2$ in pyrazoline ring, which vividly depicts the formation of pyrazoline ring. Besides this, $^{13}$C NMR and DEPT spectrum also show peaks for $–CH_2$, and –CH group, consistent with the suggested structures.

## BIOLOGICAL EVALUATION

### Cytotoxicity Study

The *in vitro* anticancer activity of the newly synthesised compounds N1-acetylated pyrazolines (**5a**–**c**), N1-phenyl pyrazolines (**6a**–**e**), 3,5-diaryl-1-carbothioamide-pyrazolines (**7a**–**e**), chalcone analogue (**3a**), and pyrazoline analogue of



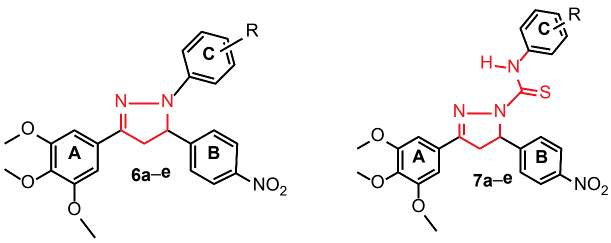

Scheme 1. Reagents and conditions: (*i*) 10 % NaOH, ethanol, rt, 12 h; (*ii*) Substituted phenylhydrazine hydrochloride, absolute ethanol, reflux, 6 h; (*iii*) $H_2NNH_2 \cdot H_2O$, absolute ethanol, reflux, 6 h; (*iv*) Substituted phenyl isothiocyanate, absolute ethanol, reflux, 60 min; (*v*) $H_2NNH_2 \cdot H_2O$, HCOOH / $CH_3COOH$ / $CH_3CH_2COOH$, reflux, 6 h.

CA-4 (**4a**) was assessed using the sulforhodamine B (SRB) assay. The anticancer drug adriamycin was used as a reference standard. All the compounds were screened against two cancer cell lines, specifically, MCF-7 (breast) and Vero (monkey kidney normal cell line). Moreover, five selected compounds from the series were also investigated for their *in vitro* anticancer activity against Hela (Human Cervical), MDA-MB-231 (Breast), and A-549 (Lung).

The screening process recorded three response parameters ($GI_{50}$, TGI, and $LC_{50}$). The $GI_{50}$ value (growth inhibitory activity) unveils the concentration of a compound triggering 50 % reduction in the net cell growth, the TGI value (cytostatic activity) reveals the concentration of a compound required for the total growth inhibition while the $LC_{50}$ value (cytotoxic activity) determines the concentration of a compound that cause the net 50 % loss of initial cells. The response parameters for all the compounds against MCF-7 and Vero (Monkey kidney normal cell line) along with the responses against Hela (Human Cervical), MDA-MB-231 (Breast), and A-549 (Lung) for selected five compounds are presented in the Table 1. The results obtained is related to the $GI_{50}$ values, accordingly, the compound's activity is classified as: inactive, >100 µM; moderate, between >30 and <100 µM; good, between >10 and <30 µM; and active, <10 µM.

The screening process recorded three response parameters ($GI_{50}$, TGI, and $LC_{50}$). The $GI_{50}$ value (growth inhibitory activity) reveals the concentration of a compound triggering a 50 % reduction in the net cell growth; the TGI value (cytostatic activity) reveals the concentration

Table 1. In vitro anticancer screening of synthesized compounds against MCF-7[(a)], Hela[(a)], MDA-MB-231[(a)], A-549[(a)] and Vero[(a)] cell line




| Entry | R | MCF-7 | | | Hela | | | MDA-MB-231 | | | A-549 | | | Vero | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $LC_{50}$[(b)] | TGI[(c)] | $GI_{50}$[(d)] | $LC_{50}$[(b)] | TGI[(c)] | $GI_{50}$[(d)] | $LC_{50}$[(b)] | TGI[(c)] | $GI_{50}$[(d)] | $LC_{50}$[(b)] | TGI[(c)] | $GI_{50}$[(d)] | $LC_{50}$[(b)] | TGI[(c)] | $GI_{50}$[(d)] |
| **3a** | – | >100 | >100 | 10.0 | >100 | >100 | 50.0 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 |
| **4a** | – | >100 | >100 | 60.0 | >100 | >100 | >100 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 |
| **5a** | H | >100 | >100 | 30.0 | NT | NT | NT | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | 50.0 |
| **5b** | $CH_3$ | >100 | >100 | 20.0 | NT | NT | NT | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | 60.0 |
| **5c** | $CH_3CH_2$ | >100 | >100 | 40.0 | NT | NT | NT | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | 50.0 |
| **6a** | 2–$CH_3$ | >100 | >100 | 10.0 | NT | NT | NT | NT | NT | NT | >100 | >100 | 20.0 | >100 | >100 | >100 |
| **6b** | 4–$CH_3$ | >100 | >100 | >100 | >100 | >100 | >100 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 |
| **6c** | 4-Cl | >100 | >100 | >100 | >100 | >100 | >100 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 |
| **6d** | 4–CN | >100 | >100 | 90.0 | NT | NT | NT | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | >100 |
| **6e** | 4-F | >100 | >100 | 10.0 | >100 | >100 | >100 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 |
| **7a** | 2–$CH_3$ | >100 | >100 | 20.0 | NT | NT | NT | NT | NT | NT | >100 | >100 | 30.0 | >100 | >100 | >100 |
| **7b** | 4–$CH_3$ | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | >100 | NT | NT | NT | >100 | >100 | >100 |
| **7c** | 4-Cl | >100 | >100 | >100 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 | >100 | >100 | >100 |
| **7d** | 4–CN | >100 | >100 | 30.0 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 | >100 | >100 | >100 |
| **7e** | 2-$OCH_3$ | >100 | >100 | 60.0 | NT | NT | NT | NT | NT | NT | >100 | >100 | >100 | >100 | >100 | >100 |
| **Adriamycin** | – | >100 | 1.0 | <0.1 | >100 | 6.0 | <0.1 | 20.0 | <0.1 | <0.1 | >100 | 1.0 | <0.1 | >100 | 1.0 | <0.1 |

[(a)] Concentrations in µM

[(b)] Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from $[(Ti - Tz) / Tz] \times 100 = -50$

[(c)] Drug concentration resulting in total growth inhibition (TGI) will calculated from $Ti = Tz$

[(d)] Growth inhibition of 50 % ($GI_{50}$) calculated from $[(Ti - Tz) / (C - Tz)] \times 100 = 50$

NT = Not tested.

of a compound required for the total growth inhibition; and the $LC_{50}$ value (cytotoxic activity) determines the concentration of a compound that causes the net 50 % loss of initial cells. The response parameters for all the compounds against MCF-7 and Vero (monkey kidney normal cell line), along with the responses against Hela (Human Cervical), MDA-MB-231 (Breast), and A-549 (Lung) for selected five compounds are presented in Table 1. The results obtained are related to the $GI_{50}$ values. Consequently, the compound's activity is classified as: inactive, >100 µM; moderate, between >30 and <100 µM; good, between >10 and <30 µM; and active, <10 µM.

The data obtained reveals that among the three categories of novel combretastatin-A4 analogues, most of the compounds exhibited significant cytotoxicity against the MCF-7 cell line, with the concentration of a compound that triggers 50 % inhibition of cell growth ($GI_{50}$). In particular, compounds **3a**, **6a**, **6e**, **5b**, **7a**, **5a**, and **7d** showed profound inhibitory effect against the MCF-7 cell line that exhibited a wide spectrum of activity with the $GI_{50}$ value ranging from 10 to 30 µM. Whereas, compounds **5c** and **7e** demonstrated moderate cytotoxicity ($GI_{50}$ = 60 µM) and the remaining compounds displayed weak cytotoxicity ($GI_{50}$ = 90 – 100 µM).

Out of the selected compounds tested against Hela (Human Cervical), MDA-MB-231 (Breast), and A-549 (Lung) cell lines, compounds **6a** and **7a** showed remarkable cytotoxicity against the A-549 (Lung) cell line with the $GI_{50}$ value ranging from 10 to 30 µM, and compound **3a** displayed moderate cytotoxicity against Hela (Human Cervical). Other compounds did not show significant inhibitory potential. Moreover, all the compounds in the series were tested on the Vero (monkey kidney) cell line, which represents the normal cell type. The results clearly show that these compounds did not show cytotoxicity, except for compounds **5a-c,** which showed moderate cytotoxicity against the normal cell line. Unlike the reference standard drug adriamycin, it exhibited remarkable cytotoxicity against the Vero (Monkey kidney) normal cell line. The results reveal that compounds **6a**, **6e,** and **7a**, **7d** selectively target cancerous cells (MCF-7, A-549 Lung) and thus can be considered as a lead for the development of an effective anticancer drug.

A similar relationship has been established for the TGI concentrations of the compounds in comparison with adriamycin, the reference standard drug. All the tested compounds appeared to be inactive against all the tested cell lines. Likewise, the $LC_{50}$ concentrations of the compounds were compared with adriamycin to get an insight into the cytotoxic effects of these compounds against all the cell lines All the compounds ($LC_{50}$ > 100 µM) like adriamycin ($LC_{50}$ > 100 µM) were found to be inactive against all the cell lines, except adriamycin did show potency against MDA-MB-231 ($LC_{50}$ > 20 µM).

The structure activity relationship (SAR) study reveals that the compound **3a** (chalcone analogue of CA-4) with the same substituent on A ring and B ring replaced with a 4-nitro-phenyl group showed good cytotoxicity against the MCF-7 cell line ($GI_{50}$ = 10.0 µM) and moderate cytotoxicity against the Hela cell line. However, the compound **4a** (pyrazoline analogue of CA-4) showed moderate cytotoxicity against the MCF cell line ($GI_{50}$= 60.0 µM) and poorly inhibited other cell lines. When the phenyl or phenyl carbothioamide group was substituted at the $N^1$ position of pyrazoline, the activity increased. Compounds **6a** and **7a** with a $-CH_3$ group at the ortho position of the ring-C of $N^1$-phenyl pyrazoline and 3,5-diaryl-1-carbothioamide-pyrazoline showed better cytotoxicity against MCF-7 and A-549 cell lines than that of **4a**. Moreover, compounds **6e** and **7d** with fluoro and cyano groups at the para position of the C-ring showed enhanced cytotoxicity against the MCF cell line. From this evidence, a general specific trend in structure and activity can be established. Since then, chalcone and pyrazoline analogues of CA-4 adopt twisted geometry[18,26–27] like that of CA-4, which is indispensable to fit into the binding site of tubulin to inhibit their polymerization. Among the pyrazoline analogues (**6a**–**e**) and (**7a**–**e**), most of the compounds appeared to be potent. Since then, a small change in the structure of the CA-4 analogue has shown a surprising effect on other biological targets.

## CONCLUSION

In conclusion, fourteen pyrazoline analogue of combretastatin A-4 (**5a**–**c**), (**6a**–**e**) and, (**7a**–**e**) were synthesised from the precursor chalcone analogue of combretastatin A-4 (**3a**). All the compounds were tested against two cancer cell lines *viz*, MCF-7 (breast) and Vero (monkey kidney normal cell line). Moreover, five compounds selected from the series were also tested for their anticancer activity against Hela (human Cervical), MDA-MB-231 (breast), and A-549 (lung) cell lines. Compounds **3a**, **6a** and **6e** demonstrated the highest cytotoxicity followed by **5b**, **7a**, **5a** and **7d** against the MCF-7 cell line**.** Compounds **6a** and **7a** also displayed remarkable cytotoxic effect against A-549 (lung) cell line. Overall, MCF-7 and A-549 (lung) cell lines are susceptible to the set of synthesized compounds. In general, the presence of 4-F/4–CN/2–$CH_3$ groups on C-ring of $N^1$-phenyl pyrazoline and 3,5-diaryl-1-carbothioamide-pyrazoline showed good cytotoxicity against MCF-7 and A-549 cell line. The outcomes of the study undoubtedly reveal that a set of compounds **6a**, **6e** and **7a**, **7d** selectively target only cancerous cells (MCF-7, A-549 Lung) and thus, can be considered a lead for the development of an effective anticancer drug.

**Acknowledgment.** The authors are grateful to Punyashlok Ahilyadevi Holkar Solapur University, Solapur, Maharashtra, 413255, India for financial support under Seed money for the Researcher project.

**Conflict of Interest.** The authors declare no potential conflicts of interest.

**Supplementary Information.** Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3859.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

## REFERENCES

[1] H. Singla, S. Kalra, P. Kheterpal, V. Kumar, A. Munshi, *Curr. Cancer Drug Target*, **2017**, 17, 344–356. https://doi.org/10.2174/1568009616666161216094026

[2] C. Avendaño, J. C. Menendez, *Medicinal chemistry of anticancer drugs*. Elsevier, **2008.**

[3] G. Bacher, T. Beckers, P. Emig, T. Klenner, B. Kutscher, *Pure Appl. Chem.,* **2001,** 73, 1459–1464. https://doi.org/10.1351/pac200173091459

[4] S. Honore, E. Pasquier, D. Braguer, *Cell. Mol. Life Sci.*, **2005**, 62, 3039–3056. https://doi.org/10.1007/s00018-005-5330-x

[5] E. A. Perez, *Mol Cancer Ther.*, **2009**, 8, 2086–2095. https://doi.org/10.1158/1535-7163.MCT-09-0366

[6] A. Jordan, L. Wilson, *Nat. Rev. Cancer*, 2**004**, 4, 253–265. https://doi.org/10.1038/nrc1317

[7] E. Pasquier, M. Kavallaris, *IUBMB Life*, **2008**, 60, 165–170. https://doi.org/10.1002/iub.25

[8] R. Cao, M. Liu, M. Yin, Q. Liu, Y. Wang, N. Huang, *J. Chem. Inf. Model*, **2012**, 52, 2730–2740. https://doi.org/10.1021/ci300302c

[9] B. Bhattacharyya, D. Panda, S. Gupta, M. Banerjee, *Med Res Rev.,* **2008,** *28*, 155–183. https://doi.org/10.1002/med.20097

[10] B. Kumar, S. Singh, I. Skvortsova, V. Kumar, *Curr. Med. Chem.*, **2017**, 24, 4729–4752. https://doi.org/10.2174/0929867324666170331123648

[11] C. J. Mooney, G. Nagaiah, P. Fu, J. K. Wasman, M. M. Cooney, P. S. Savvides, J. A. Bokar, A. Dowlati, D. Wang, S. S. Agarwala, *Thyroid*, **2009**, 19, 233–240. https://doi.org/10.1089/thy.2008.0321

[12] S. M. Galbraith, R. J. Maxwel, M. A. Lodge, G. M. Tozer, J. Wilson, N. J. Taylor, J. J. Stirling, L. Sena, A. R. Padhani, G. S. Rustin, *J. Clin. Oncol.*, 2003, 15, 2831–2842. https://doi.org/1200/JCO.2003.05.187

[13] J. Ruprich, A. Prout, J. Dickson, B. Younglove, L. Nolan, K. Baxi, R. LeBlanc, L. Forrest, P. Hills, H. Holt, H. Mackay, T. Brown, S. Mooberry, M. Lee, *Lett. Drug Des. Disc.,* 2007, 4, 144–148. https://doi.org/10.2174/157018007779422442

[14] C. Congiu, V. Onnis, L. Vescil, M. Castorina, C. Pisano, *Bioorg. Med. Chem.*, 2010, 18, 6238–6248. https://doi.org/10.1016/j.bmc.2010.07.037

[15] G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca, A. A. Genazzani, *J. Med. Chem.*, **2006**, 49, 3033–3044. https://doi.org/10.1021/jm0512903

[16] R. Romagnoli, P. G. Baraldi, O. Cruz-Lopez, C. C. Lopez, M. D. Carrion, A. Brancale, E. Hamel, L. Chen, R. Bortolozzi, G. Basso and G. Viola, *J. Med. Chem*. **2010**, 53, 4248–4258. https://doi.org/10.1021/jm100245q

[17] A. Chaudari, S. N. Pandeya, P. Kumar, P. P. Sharma, S. Gupta, N. Soni, K. K. Verma, G. Bhardwaj, *Mini-Rev. Med. Chem.*, **2007**, 12, 1186–1205. https://doi.org/10.2174/138955707782795647

[18] B. L. Flynn, G. P. Flynn, E. Hamel, M. K. Jung, *Bioorg. Med. Chem. Lett.* **2001**, 11, 2341–2343. https://doi.org/10.1016/s0960-894x(01)00436-x

[19] S. N. Shringare, H. V. Chavan, P. S. Bhale, S. B. Dongare, Y. B. Mule, N. D. Kolekar, B. P. Bandgar, *Croat. Chem. Acta.* **2018**, 91, 357–366. https://doi.org/10.5562/cca3393

[20] X. Zhang, S. Raghavan, M. Ihnat, E. Hamel, C. Zammiello, A. Bastian, S. L. Mooberry, A. Gangjee, *Bioorg. Med. Chem.* **2015**, 23, 2408–2423. https://doi.org/10.1016/j.bmc.2015.03.061

[21] S. Zheng, Q. Zhong, M. Mottamal, Q. Zhang, C. Zhang, E. LeMelle, H. McFerrin, G. Wang, *J. Med. Chem*. **2014**, 57, 3369–3381. https://doi.org/10.1021/jm500002k

[22] S. N. Shringare, H. V. Chavan, P. S. Bhale, S. B. Dongare, Y. B. Mule, S. B. Patil, B. P. Bandgar, *Med. Chem. Res*. **2018**, 27, 1226–1237. https://doi.org/10.1007/s00044-018-2142-8

[23] P. S. Bhale, H. V. Chavan, S. B. Dongare, S. N. Shringare, Y. B. Mule P. B. Choudhari, B. P. Bandgar, *Curr. Bioactive. Compounds*, **2018**, 14, 299–308. https://doi.org/10.2174/1573407213666170428112855

[24] V. Vichai, K. Kirtikara, *Nat. Protoc*. **2006**, 1, 1112–1116. https://doi.org/10.1038/nprot.2006.179

[25] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J Natl Cancer Inst*. **1990**, 82, 1107–1112. https://doi.org/10.1093/jnci/82.13.1107

[26] S. Ducki, R. Forrest, J. A. Hadfield, A. Kendall, N. J. Lawrence, A. T. McGown, D. Rennison, *Bioorg. Med. Chem. Lett*. **1998**, 8, 1051–1056. https://doi.org/10.1016/s0960-894x(98)00162-0

[27] M. Johnson, B. Younglove, L. Lee, R. LeBlanc, H. Jr. Holt, P. Hills, H. Mackay, T. Brown, S. L. Mooberry, M. Lee, *Bioorg. Med. Chem. Lett.* **2007**, 17, 897–5901. https://doi:10.1016/j.bmcl.2007.07.105