View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta 2018, *91*(3), 357–366
Published online: November 7, 2018
DOI: 10.5562/cca3393

HKD 1926

Synthesis and Pharmacological Evaluation of Pyrazoline and Pyrimidine Analogs of Combretastatin-A4 as Anticancer, Anti-inflammatory and Antioxidant Agents

Sadanand N. Shringare,[1] Hemant V. Chavan,[2,*] Pravin S. Bhale,[1] Sakharam B. Dongare,[1] Yoginath B. Mule,[1] Nishikant D. Kolekar,[1] Babasaheb P. Bandgar[1]

[1] Medicinal Chemistry Research Laboratory, School of Chemical Sciences, Solapur University, Solapur, Maharashtra, 413255, India

[2] Department of Chemistry, A.S.P. College Devrukh, (University of Mumbai), Dist-Ratnagiri-415 804, Maharashtra, India

* Corresponding author's e-mail address: hemantchavan.sus@rediffmail.com

RECEIVED: July 2, 2018 ⁎ REVISED: September 14, 2018 ⁎ ACCEPTED: September 17, 2018

Abstract: A library of 3,5-diaryl-1-carbothioamide-pyrazoline (**5a–j**), N^1-phenyl sulfonyl pyrazoline (**6a–e**) and pyrimidine (**7a)** analogs of combretastatin-A4 were synthesized and evaluated for their *in vitro* anticancer, anti-inflammatory and antioxidant activity. Results of *in vitro* assay against human breast cancer cell line (MCF-7) showed several compounds endowed with significant cytotoxicity compared to the adriamycin, a standard anticancer drug. Among the compounds synthesized, **7a** was found to possess significant antiproliferative activity (GI_{50} < 0.1 μM) against the MCF-7 cell line as good as adriamycin (GI_{50} < 0.1 μM) whereas, compounds **6c**, **5j** and **5g** also displayed good cytotoxicity (GI_{50} = 25.3–42.6 μM). Besides this, most active compound **7a** was also evaluated against human myeloid leukemia cell line K562 and the remarkable result was obtained with GI_{50} < 0.1 μM, comparable to that of adriamycin (GI_{50} < 0.1 μM). In addition, all the synthesized compounds were evaluated for their anti-inflammatory and antioxidant activity. The percent inhibition studies revealed that most of the compounds were found to possess substantial anti-inflammatory and antioxidant activities.

Keywords: pyrazoline, pyrimidine, combretastatin, anticancer, anti-inflammatory, antioxidant.

INTRODUCTION

CANCER is a serious and dreadful disease, difficult to alleviate. It is clearly understood that cancer is a disease of the cell cycle, a complex process regulated by four consecutive phases: gap 1 (G1), DNA-synthesis (S), gap 2 (G2) and mitosis (M). The failure to control checkpoints in the cell cycle leads to uncontrolled proliferation of cell.[1] Chemotherapy is still one of the ways for the treatment of cancer. The currently available anticancer agents manifested undesirable side effects such as low bioavailability, toxicity, and drug-resistance.[2] Thus, the discovery of new, effective and selective anticancer agents is still a challenge in medicinal chemistry. Nevertheless, understanding the molecular mechanism involved in cancers can help to procure novel anticancer agent. One such approach is to target microtubule, a dynamic structure that elongates or shrinks with the addition or exclusion of tubulin proteins.[3] It is also an important cytoskeletal filament crisscrossing the cytoplasm of all the eukaryotic cells and perform a vital cellular function such as separation of the chromosome during mitosis, shape maintenance and vesicle transport. As a result, agents that interact at the interface of α,β-dimers of tubulin, that is, at the colchicine binding site, inhibit tubulin assembly into microtubules. Combretastatins, derived from the bark of the African willow tree, *Combretum caffrum,*[4] have received considerable importance due to their ability to prevent cancer cell growth. Combretastatin-A4 (**1, CA-4,** Figure 1) in particular, is an effective antivascular and antimitotic agent, which inhibit tubulin polymerization by binding to colchicine binding site.[5] Consequently, lack of microtubule in the metaphase of the cell cycle halts mitotic spindle formation.[6] Besides, it alters endothelial cell structure and vascular permeability, resulting in vascular collapse and tumor necrosis.[2,7] Despite the potent cytotoxic and anti-tubulin *in vitro* efficacy, CA-4 does come with

This work is licensed under a Creative Commons Attribution 4.0 International License.

major limitation of high liphophilicity and low solubility in aqueous media to develop it as a possible anti-tumor agent.[8] The aforementioned physicochemical restriction and the simple structural template of combretastatin-A4 has led to design many structural analogs to improve *in vivo* efficacy, such as the water-soluble phosphate prodrugs of CA-4, an amino analog (**2,** Figure 1) and an amino acid derivatives,[9] which have shown remarkable potency. Furthermore, SAR studies reveal that the cis-configuration of the two benzene rings and 3,4,5-trimethoxy substituent on the A-ring of CA-4 are requisite for potent cytotoxicity.[10] This indeed has promoted researchers across the world to focus on the design of CA-4 analogs by altering the bridgehead linker and the B-ring of the CA-4 in order to augment the bioavailability and antitumor activity. A broad range of structural analogs of CA-4 have been reported, which include substitution on B-ring in the combretastatin framework with different heterocycles [11] and replacing the stilbene bridgehead linker with different functional groups, for example, α,β-unsaturated ketone,[12] and 1,3-disubstituted three-carbon linker.[13]

Pyrazolines are the rich class of five-member heterocycles comprise a wide range of pharmacological activities including anti-inflammatory,[14,15] antitumor,[16] MAO-B inhibitors[17] and antioxidant activity.[18] Recently, pyrazoline bearing 3,4,5-trimethoxy phenyl moiety reported as a potent anti-inflammatory agent (**3,** Figure 1).[19] On the other hand, 2,4,5-trimethoxy chalcones, their analogues 2,4,5-trimethoxy-2′,5′-dihydroxychalcone, and hydrazone bearing a 3,4,5-trimethoxy benzyl have shown superior DPPH radical scavenging activities (**4,** Figure 1).[20] Taking into consideration the aforementioned reports, and in continuation of our earlier efforts on development of anticancer, antioxidant and anti-inflammatory agents,[21,22] we herein, intended to report combretastatin analogs by altering stilbene bridgehead linker (Scheme 1).

EXPERIMENTAL

Materials and Methods

All the chemicals and solvents used were of analytical grade and used without purification. All the reactions were monitored by thin layer chromatography, (TLC silica gel 60 F_{254} by Merck) and were visualized under a UV lamp and using iodine vapors. The melting points were ascertained with a digital thermometer and are uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer). ^{1}H NMR spectra were recorded on Bruker DRX FT spectrometer at 200 MHz and 400 MHz using $CDCl_3$ / DMSO-d6 as a solvent. Chemical shift values recorded are mentioned in parts per million (ppm) and observed downfield from TMS, while coupling constants (*J*) are referred to in hertz (Hz).

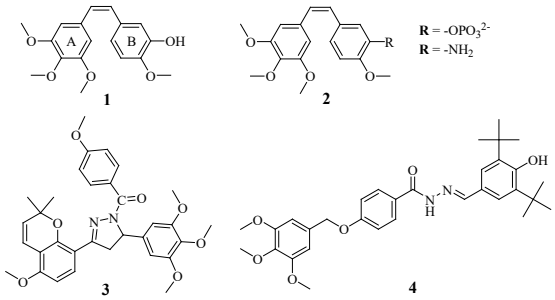


Figure 1. Some biologically active methoxylated derivatives.

Abbreviations used in the splitting pattern were as follows: s = singlet, d = doublet, t = triplet, q = quartet, qu = quintet and m = multiplet. The mass spectra were determined on Shimadzu LCMS-2010 EV instrument.

Synthesis

GENERAL PROCEDURE FOR THE PREPARATION OF 3,5-DIARYL-1-CARBOTHIOAMIDE-PYRAZOLINE (5a–j)

To a suspension of 5-(4,5-dihydro-3-(3,4,5-trimethoxyphenyl)-1H-pyrazol-5-yl)-2-methoxyphenol **4a** (1 mmol) in 5 mL absolute ethanol, substituted phenyl isothiocynate (1 mmol) was added and the mixture was stirred at reflux. The progress of the reaction was monitored by TLC. After completion of reaction (1h), the reaction mixture was allowed to cool at room temperature. The solid precipitated was filtered, washed with hot ethanol (2x3mL), and dried under vacuum to obtain title compounds (**5a–j**).

5-(3-Hydroxy-4-methoxyphenyl)-*N*-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5a)

Yield: 90 %; MP: 218 °C; MF: $C_{27}H_{29}N_3O_6S$; IR (KBr, cm^{-1}): 3390 (OH), 3322 (NH), 2926 (C=C–H), 2834 (C–H), 1594 (C=N), 1568 (C=C), 1348 (C=S), 1220 and 1069 (C–O); ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 3.252 (d, 1H, *J* = 9.2 Hz, –CH_2–pyrazoline), 3.753–3.704 (m, 9H, OCH_3), 3.802–3.704 (m, 1H, –CH_2–pyrazoline), 3.845 (s, 6H, OCH_3), 5.874 (d,1H, *J* = 4.6 Hz; –CH–pyrazoline), 6.589 (s, 2H, ArH), 6.852 (d, 1H, *J* = 4.4Hz, ArH), 6.914 (d, 2H, *J* = 4.2 Hz, ArH), 7.256 (s, 2H, ArH), 7.341 (d, 2H, *J* = 4.2 Hz, ArH), 8.985 (s, 1H, ArOH), 9.985 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$):): ^{TM} = 42.8, 55.44,56, 56.34, 61, 63, 104.23, 111, 111.40, 114, 118, 126.21, 127.23, 132, 135.36, 141, 146.96, 146.99, 153.46, 155, 158, 175; MS: *m* / *z* 524.05 (M+H).

N-(4-Fluorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5b)

Yield: 92 %; MP: 270 °C; MF: $C_{26}H_{26}N_3O_5FS$; IR (KBr, cm^{-1}): 3386 (OH), 3292 (NH), 2932 (C=C–H), 2835 (C–H), 1594

(C=N), 1568 (C=C), 1309 (C=S), 1204 and 1030 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM} = 3.274 (d, 1H, *J* = 9.2 Hz, –CH$_2$–pyrazoline), 3.709 (s, 3H, OCH$_3$), 3.725 (s, 3H, OCH$_3$), 3.850 (s, 6H, OCH$_3$), 3.893–3.796 (m, 1H, –CH$_2$–pyrazoline), 5.883 (d, 1H, *J* = 4.6 Hz, –CH–pyrazoline), 6.593 (s, 2H, ArH), 6.856 (d, 1H, *J* = 4.4Hz, ArH), 7.189 (t, 2H, *J* = 4.4, 4.2 Hz, ArH), 7.263 (s, 2H, ArH), 7.498 (t, 2H, *J* = 4.2, 2.4 Hz, ArH), 8.994 (s, 1H, ArOH), 10.089 (s, 1H, NH); ^{13}C NMR (100 MHz, CDCl$_3$): ^{TM}= 42.8, 56, 56.30, 61.0, 63.0, 104.30, 111.10, 112, 115.13, 115.36, 117, 127.17, 127.25, 135, 140.66,146.28, 153.38, 155.41, 174.35; MS: *m* / *z* 511 (M+H).

_N_-(2,4-Dichlorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5c)

Yield: 77 %; MP: 194 °C: MF: C$_{26}$H$_{25}$N$_3$O$_5$Cl$_2$S; IR (KBr, cm^{-1}): 3401 (OH), 3296 (NH), 2929 (C=C–H), 1595 (C=N), 1569 (C=C), 1333 (C=S), 1237 and 1031 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM}= 3.302 (d, 1H, *J* = 12.6 Hz, –CH$_2$–pyrazoline), 3.708 (s, 3H, OCH$_3$), 3.726 (s, 3H, OCH$_3$), 3.842 (s, 6H, OCH$_3$), 3.897 (t, 1H, *J* = 5.8, 3.6 Hz, –CH$_2$–pyrazoline), 5.86 (d, 1H, J = 4.8 Hz, –CH–pyrazoline), 6.601 (s, 2H, ArH), 6.853 (d, 1H, *J* = 4.2 Hz, ArH), 7.240 (s, 2H, ArH), 7.451 (d, 1H, *J* = 3.6 Hz, ArH), 7.667 (d, 1H, *J* = 4.4 Hz, ArH), 7.721 (s, 1H, ArH), 8.986 (s, 1H, ArOH), 10.039 (s, 1H, NH); MS: *m* / *z* 563 (M+H).

_N_-(4-Cyanophenyl)-5-(3-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5d)

Yield: 84 %; MP: 198 °C; MF: C$_{27}$H$_{26}$N$_4$O$_5$S; IR (KBr, cm^{-1}): 3398 (OH), 3311 (NH), 2993 (C=C–H), 2835 (C–H), 2227 (C≡N), 1603 (C=N), 1580 (C=C), 1309 (C=S), 1225 and 1030 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz):): ^{TM}= 3.314 (d, 1H, *J* = 6.4 Hz –CH$_2$–pyrazoline), 3.716 (s, 3H, OCH$_3$), 3.724 (s, 3H, OCH$_3$), 3.857 (s, 6H, OCH$_3$), 3.907 (t, 1H, *J* = 5.6, 3.6 Hz, –CH$_2$–pyrazoline), 5.918 (s, 1H, *J* = 4.4 Hz, –CH–pyrazoline), 6.596 (s, 2H), 6.861 (d, 1H, *J* = 4.4 Hz, ArH), 7.274 (s, 2H, ArH), 7.802 (d, 2H, *J* = 4.2 Hz, ArH), 7.945 (d, 2H, *J* = 4.2 Hz, ArH), 8.995 (s, 1H, ArOH), 10.343 (s, 1H, NH); ^{13}C NMR (100 MHz, CDCl$_3$): ^{TM}= 43, 56, 56.42, 61.0, 63.25, 105, 107, 112, 113, 117, 119, 124, 126.05, 132.27, 135, 141, 144, 146.78, 147.08, 153.35, 156.31, 172.47; HRMS: *m* / *z* 519.1708 (M+H).

5-(3-Hydroxy-4-methoxyphenyl)-_N_-_p_-tolyl-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5e)

Yield: 86 %; MP: 250 °C; MF: C$_{27}$H$_{29}$N$_3$O$_5$S; IR (KBr, cm^{-1}): 3401 (OH), 3300 (NH), 2922 (C=C–H), 1595 (C=N), 1570 (C=C), 1307 (C=S), 1223 and 1030 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM}= 2.297 (s, 3H, CH$_3$), 3.258 (t, 1H, *J* = 1.2, 8 Hz, –CH$_2$–pyrazoline), 3.705 (s, 3H, OCH$_3$), 3.722 (s, 3H, OCH$_3$), 3.846 (s, 6H, OCH$_3$), 3.863 (d, 1H, *J* = 7 Hz, –CH$_2$–pyrazoline), 5.885 (d, 1H, *J* = 4.4 Hz, –CH–pyrazoline), 6.591 (s, 2H, ArH), 6.852 (d, 1H, *J* = 4.2 Hz, ArH), 7.154 (d, 2H, *J* = 4.0 Hz, ArH), 7.258 (s, 2H, ArH), 7.364 (d, 2H, *J* = 4.0 Hz, ArH), 8.984 (s, 1H, ArOH), 10.015 (s, 1H, NH); MS: *m* / *z* 508 (M+H).

5-(3-Hydroxy-4-methoxyphenyl)-_N_-_o_-tolyl-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5f)

Yield: 92 %; MP: 242 °C; MF: C$_{27}$H$_{29}$N$_3$O$_5$S: IR (KBr, cm^{-1}): 3395 (OH), 3294 (NH), 2930 (C=C–H), 1595 (C=N), 1570 (C=C), 1316 (C=S), 1213 and 1033 (C–O): ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM}= 2.238 (s, 3H, –CH$_3$), 3.247 (d, 1H, *J* = 9.2 Hz, –CH$_2$–pyrazoline), 3.699 (s, 3H, OCH$_3$), 3.727 (s, 3H, OCH$_3$), 3.836 (s, 6H, OCH$_3$), 3.884–3.836 (m, 1H, –CH$_2$–pyrazoline), 5.872 (d, 1H, *J* = 4.4 Hz, –CH–pyrazoline), 6.594 (s, 2H, ArH), 6.854 (d, 1H, *J* = 4.0 Hz, ArH), 7.250–7.192 (m, 6H, ArH), 8.974 (s, 1H, ArOH), 9.931 (s, 1H, NH); MS: *m* / *z* 508 (M+H).

5-(3-Hydroxy-4-methoxyphenyl)-_N_-(2-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5g)

Yield: 92 %; MP: 238 °C; MF: C$_{27}$H$_{29}$N$_3$O$_6$S; IR (KBr, cm^{-1}): 3420 (OH), 3292 (NH), 2929 (C=C–H), 2852 (C–H), 1595 (C=N), 1570 (C=C), 1316 (C=S), 1212.68 and 1024 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM}= 3.318–3.274 (m, 1H, –CH$_2$–pyrazoline), 3.894–3.715 (m, 15H, OCH$_3$), 3.922–3.859 (m, 1H, –CH$_2$–pyrazoline), 5.892 (d, 1H, J = 5.6Hz, –CH–pyrazoline), 6.591 (s, 2H, ArH), 6.948–6.845 (m, 2H, ArH), 7.191–7.080 (m, 4H, ArH), 8.146 (d, 1H, *J* = 4.0 Hz, ArH), 8.962 (s, 1H, ArOH), 9.923 (s, 1H, NH); ^{13}C NMR (CDCl$_3$, 100 MHz): ^{TM}= 42.7, 56, 56.14, 61.0, 63, 104.14, 110.25, 111.0, 112.0, 117.14, 120.4, 121.7, 124.4, 126.34, 128.54, 135.21, 140.41, 146.19, 146.27, 149.8, 153.4, 154.45, 172.4; HRMS: 546.1669 (M+Na).

_N_-(4-Chlorophenyl)-5-(3-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5h)

Yield: 87 %; MP: 268 °C; MF: C$_{26}$H$_{26}$N$_3$O$_5$ClS; IR (KBr, cm^{-1}): 3390 (OH), 3300 (NH), 2930 (C=C–H), 2852 (C–H), 1595 (C=N), 1569 (C=C), 1335 (C=S), 1224 and 1030 (C–O); ^{1}H NMR (DMSO-d$_6$, 200 MHz): ^{TM} = 3.333–3.259 (m, 1H, –CH$_2$–pyrazoline), 3.709 (s, 3H, OCH$_3$), 3.723 (s, 3H, OCH$_3$), 3.851 (s, 6H, OCH$_3$), 3.875 (d, 1H, *J* = 9.6 Hz, –CH$_2$–pyrazoline), 5.889 (d, 1H, *J* = 4.8 Hz, –CH–pyrazoline), 6.587 (s, 2H, ArH), 6.854 (d, 2H, *J* = 4.2 Hz, ArH), 7.263 (s, 2H, ArH), 7.404 (d, 2H, *J* = 4.2 Hz, ArH), 7.579 (d, 2H, *J* = 4.2 Hz, ArH), 8.988 (s, 1H, ArOH), 10.131 (s, 1H, NH); MS: *m* / *z* 529 (M+H).

5-(3-Hydroxy-4-methoxyphenyl)-_N_-(4-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5i)

Yield: 89 %; MP: 278 °C; MF: C$_{26}$H$_{26}$N$_4$O$_7$S; IR (KBr, cm^{-1}): 3381 (OH), 3933 (C=C–H), 1594 (C=N), 1571 (C=C), 1310

(C=S), 1233 and 1021 (C–O), 1504 (NO_2 asym), 1364 (NO_2 sym); ^{1}H NMR (DMSO-d_6, 200 MHz): ^{TM}= 3.34 (d, 1H, *J* = 4.4 Hz, –CH_2–pyrazoline), 3.724 (s, 6H, OCH_3), 3.862 (s, 6H, OCH_3), 3.946–3.90 (m, 1H, –CH_2–pyrazoline), 5.934 (d, 1H, *J* = 5.2 Hz, –CH–pyrazoline), 6.607 (s, 2H, ArH), 6.866 (d, 1H, *J* = 4.2 Hz, ArH), 7.285 (s, 2H, ArH), 8.049 (d, 2H, *J* = 4.2 Hz, ArH), 8.222 (d, 2H, *J* = 4.2 Hz, ArH), 8.998 (s, 1H, ArOH), 10.480 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): ^{TM}= 42.79, 56, 56.44, 61.0, 63.32, 112, 113, 117, 123, 124, 126, 135, 141, 143.31, 146, 147, 153.36, 157, 172.29; HRMS: *m* / *z* 539.1604 (M+H).

5-(3-Hydroxy-4-methoxyphenyl)-*N*-phenyl-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5j)

Yield: 86 %; MP: 252°C; MF: $C_{26}H_{27}N_3O_5S$: IR (KBr, cm^{-1}): 3378 (OH), 2930 (C=C–H), 1592 (C=N), 1567 (C=C), 1309 (C=S), 1227 and 1033 (C–O); ^{1}H NMR (DMSO-d_6, 200 MHz): ^{TM}= 3.272 (d, 1H, *J* = 9.4 Hz, –CH_2–pyrazoline), 3.708 (s, 3H, OCH_3), 3.725 (s, 3H, OCH_3), 3.794 (s, 6H, OCH_3), 3.896–3.665 (m, 1H, –CH_2–pyrazoline), 5.899 (d, 1H, *J* = 5Hz, –CH–pyrazoline), 6.599 (s, 2H, ArH), 6.857 (d, 1H, *J* = 4.2Hz, ArH), 7.187 (t, 1H, *J* = 3.4Hz, *J* = 3.6Hz, ArH), 7.265 (s, 2H, ArH), 7.354 (t, 2H, *J* = 3.6Hz, 3.8Hz, ArH), 7.519 (d, 2H, *J* = 3.6Hz, ArH), 8.984 (s, 1H, ArOH), 10.086 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): ^{TM} = 42.67, 56, 56.47, 61.0, 63.34,105.12, 112.23, 113.02, 117, 125.40, 126.15, 127, 128.35, 136, 140.29, 146.96, 147.06, 153.39, 155.61, 174; MS: *m* / *z* 494 (M+1).

GENERAL PROCEDURE FOR THE PREPARATION OF N^1-PHENYL SULFONYLPYRAZOLINE (6a–e)

To a suspension of 5-(4,5-dihydro-3-(3,4,5-trimethoxyphenyl)-1H-pyrazol-5-yl)-2-methoxyphenol **4a** (1 mmol) in 5 mL absolute ethanol, substituted phenyl sulphonyl chloride (1 mmol) was added and the mixture stirred at reflux. The progress of the reaction was monitored by TLC. After completion of reaction (1 h), the mixture was allowed to cool at room temperature. The solid precipitated was filtered, washed with hot ethanol (2 × 3mL), and dried under vacuum to obtain title compounds (**6a–e**).

2-Methoxy-5-(1-(2-nitrophenylsulfonyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol (6a)

Yield: 80 %; MP: 210 °C; MF: $C_{25}H_{25}N_3O_9S$; IR (KBr, cm^{-1}): 3370 (OH), 2929 (C=C–H), 2837 (C–H), 1575 (C=N), 1538 (C=C), 1178 and 1057 (C–O), 1369 (S=O asym), 1178 (S=O sym), 1509 (NO_2 asym), 1324 (NO_2 sym); ^{1}H NMR (400 MHz, $CDCl_3$): ^{TM}= 3.210 (dd, 1H, *J* = 7.2 Hz, J = 6.8 Hz, –CH_2–pyrazoline), 3.712 (dd, 1H, *J* = 2.8, 11.2 Hz, –CH_2–pyrazoline), 3.907–3.898 (m, 12H, OCH_3), 5.409 (dd, 1H, *J* = 6.8 Hz, *J* = 7.2 Hz, –CH_2–pyrazoline), 5.649 (s, 1H, ArOH), 6.828 (d, 2H, *J* = 8 Hz, ArH), 6.961–6.926 (m, 4H, ArH), 7.711–7.561 (m, 3H, ArH), 8.094 (d, 1H, J = 7.2Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): ^{TM} = 43.8, 56.0, 56.31, 61.0, 64.3, 104.4, 111.0, 113.0, 118.3, 123.63, 125.9, 129.7, 131.05, 132.0, 133.7, 134.08, 140.5, 146.25, 147.0, 148.7, 153.3, 156.8; MS: *m* / *z* 544 (M+1).

5-(1-(4-Chlorophenylsulfonyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (6b)

Yield: 88 %; MP: 160 °C; MF: $C_{25}H_{25}N_2O_7ClS$; IR (KBr, cm^{-1}): 3387 (OH), 2931 (C=C–H), 1572 (C=N), 1509 (C=C), 1169 and 1056 (C–O), 1362 (S=O asym), 1169 (S=O sym); ^{1}H NMR (400 MHz, $CDCl_3$, in ppm): ^{TM}= 3.150 (dd, 1H, *J* = 8, 8 Hz, –CH_2–pyrazoline), 3.548 (dd, 1H, *J* = 11.2, 11.2 Hz, –CH_2–pyrazoline), 3.916 (s, 6H, OCH_3), 3.906 (s, 6H, OCH_3), 4.911 (dd, 1H, *J* = 8, 8 Hz, –CH_2–pyrazoline), 5.640 (s, 1H, ArOH), 6.881–6.791 (m, 3H, ArH), 6.927 (s, 2H, ArH), 7.447–7.413 (m, 2H, ArH), 7.786–7.752 (m, 2H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): ^{TM} = 43.8, 56.01, 56.4, 61.0, 65.0, 104.3, 110.54, 112.9, 118.64, 126.0, 129.01, 129.7, 133.4, 134.7, 139.7, 140.54, 145.81, 146.54, 153.35, 156.7; HRMS: *m* / *z* 533.1152 (M+H).

5-(1-(4-Chloro-3-fluorophenylsulfonyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (6c)

Yield: 74 %; MP: 156 °C; MF: $C_{25}H_{24}N_2O_7ClFS$: IR (KBr, cm^{-1}): 3402 (OH), 2932 (C=C–H), 1575 (C=N), 1511 (C=C), 1178 and 1087 (C–O), 1369 (S=O asym), 1233 (S=O sym); ^{1}H NMR (400 MHz, $CDCl_3$): ^{TM} = 3.206 (dd, 1H, *J* = 7.2, 7.2 Hz, –CH_2–pyrazoline), 3.617 (dd, 1H, *J* = 11.2, 11.2 Hz, –CH_2–pyrazoline), 3.960–3.911 (m, 12H, OCH_3), 5.041 (dd, 1H, *J* = 6.8, 7.2 Hz, –CH_2–pyrazoline), 5.628 (s, 1H, ArOH), 6.850–6.716 (m, 3H, ArH), 6.939 (s, 2H, ArH), 7.201 (t, 1H, J = 8.4, 8.8 Hz, ArH), 7.765–7.702 (m, 2H, ArH); HRMS: *m* / *z* 551.1050 (M+H).

2-Methoxy-5-(1-(4-nitrophenylsulfonyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol (6d)

Yield: 82%; MP: 208 °C: MF: $C_{25}H_{25}N_3O_9S$: IR (KBr, cm^{-1}): 3403 (OH), 2929 (C=C–H), 1594 (C=N), 1568 (C=C), 1171 and 1023 (C–O), 1345 (S=O asym), 1171 (S=O sym), 1503 (NO_2 asym), 1345(NO_2 sym): ^{1}H NMR (400 MHz, $CDCl_3$, in ppm): ^{TM}= 3.118 (dd, 1H, *J* = 9.6, 11.2 Hz, –CH_2–pyrazoline), 3.548 (m, 1H, –CH_2–pyrazoline), 3.840–3.780 (m, 12H, OCH_3), 4.980 (dd, 1H, *J* = 9.2, 10.8 Hz, –CH_2–pyrazoline), 6.639–6.583 (m, 3H, ArH), 6.871–6.843 (m, 2H, ArH), 7.259 (s, 1H, ArOH), 7.841–7.791 (m, 2H, ArH), 8.166–8.115 (m, 1H, ArH); HRMS: *m* / *z* 544.1390 (M+H).

5-(1-(2-chlorophenylsulfonyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (6e)

Yield: 77 %; MP: 229 °C; MF: $C_{25}H_{25}N_2O_7ClS$: IR (KBr, cm^{-1}): 3381 (OH), 2939 (C=C–H), 2836 (C–H), 1575 (C=N), 1510

(C=C), 1176 and 1057 (C–O), 1367 (S=O asym), 1176 (S=O sym); ^{1}H NMR (400 MHz, $CDCl_3$): ^{TM}= 3.172 (dd, 1H, *J* = 7.6 Hz, *J* = 7.6 Hz, –CH_2–pyrazoline), 3.647 (dd, 1H, *J* = 11.2, 11.6 Hz, –CH_2–pyrazoline), 3.877–3.852 (m, 12H, OCH_3), 5.351 (dd, 1H, *J* = 7.6, 8.0 Hz, –CH–pyrazoline), 6.182 (s, 1H, ArOH), 6.79 (d, 1H, *J* = 8.0 Hz, ArH), 6.893 (d, 4H, *J* = 10.8 Hz, ArH), 7.370–7.330 (m, 1H, ArH), 7.492–7.438 (m, 2H, ArH), 8.052 (d, 1H, *J* = 8.0 Hz, ArH); HRMS: *m* / *z* 533.1148(M+H).

GENERAL PROCEDURE FOR THE PREPARATION OF PYRIMIDINE DERIVATIVE (7a)

To a suspension of (*E*)-3-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **3a** (1 mmol) in 5 mL absolute ethanol was added 10 % sodium hydroxide (NaOH) under ice cold condition and stirred for 5 min. Guanidine hydrochloride (1 mmol) was added in one portion and the mixture stirred at reflux. The progress of the reaction was monitored by TLC. After completion of reaction (24 h), the reaction mixture was poured in ice-cold water, neutralized with dilute HCl until precipitation occurs. The precipitate so obtained was filtered, washed with water and purified by column chromatography using hexane:ethyl acetate (7 : 3) to afford title compound **7a.**

5-(2-Amino-6-(3,4,5-trimethoxyphenyl)pyrimidin-4-yl)-2-methoxyphenol (7a)

Yield: 77 %; MP: 202 °C; MF: $C_{20}H_{21}N_3O_5$: IR (KBr, cm^{-1}): 3496 and 3394 (NH), 2933 (C=C–H), 1603 (C=N), 1573 (C=C), 1219 and 1022 (C–O); ^{1}H NMR (DMSO-d_6, 200 MHz): ^{TM} = 3.350 (s, 3H, OCH_3), 3.848 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3), 6.602 (s, 2H, –NH_2), 7.032 (d, 1H, *J* = 4 Hz, ArH), 7.478 (s, 2H, ArH), 7.565 (s, 1H, ArH), 7.690 (s, 1H, ArH), 7.710 (s, 1H, Pyrazole–H), 9.162 (s, 1H, ArOH); ^{13}C NMR ($CDCl_3$, 100 MHz): ^{TM} = 56.01, 56.28, 60.95, 103.41, 104.3, 110.72, 113.51, 119.43, 130.83, 133.35, 140.07, 146.0, 149.02, 153.41, 163.4, 165.51, 165.63; HRMS: 384.1554 (M+H).

Anticancer Activity

THE PROCEDURE OF THE SRB-ASSAY

Cytotoxic potencies in cancer cell lines MCF-7 and K562 were carried by sulforhodamine B (SRB) assay method. [23] Tumor cells (human breast cancer cell line MCF-7) were grown in tissue culture flasks in growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10 % fetal calf serum, 100 mg mL^{-1} streptomycin, and 100 units mL^{-1} penicillin) at 37 °C under the atmosphere of 5 % CO_2 and 95 % relative humidity employing a CO_2 incubator. The cells at the sub-confluent stage were harvested from the flask by treatment with trypsin (0.05 % trypsin in PBS containing 0.02 % EDTA) and placed in growth medium. The cells with more than 97 % viability (trypan blue exclusion) were used for cytotoxicity studies. An aliquot of 100 mL of cells was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for one day at 37 °C in a CO_2 incubator as mentioned above. The test materials at different concentrations were then added to the wells and cells were further allowed to grow for another 48h. Suitable blanks and positive controls were also included. Each test was performed in triplicate. The cell growth was stopped by gently layering of 50 mL of 50 % trichloroacetic acid. The plates were incubated at 4 °C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipetted out and discarded. The plates were washed five times with doubly distilled water to remove TCA, growth medium, *etc* and were air-dried. 100 mL of SRB solution (0.4 % in 1 % acetic acid) was added to each well and the plates were incubated at ambient temperature for half an hour. The unbound SRB was quickly removed by washing the wells five times with 1 % acetic acid. Plates were air dried, tris-buffer (100 mL of 0.01 M, pH 10.4) was added to all the wells and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was measured on ELISA reader at 540 nm. The cell growth in the absence of any test material was considered 100 % and in turn, growth inhibition was calculated. GI_{50} values were determined by regression analysis.

Antioxidant Activity

DPPH RADICAL SCAVENGING ACTIVITY

The ability of compounds to scavenge DPPH radical was assessed using Ramanathan Sambath Kumar *et al* method[24] with modification. Briefly, 1 mL of synthesized compounds as 1 mM was mixed with 3.0 mL DPPH (0.5 mmol L^{-1} in methanol), the resultant absorbance was recorded at 517 nm after 30 min incubation at 37 °C. The percentage of scavenging activity was derived using the following formula,

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is absorbance of DPPH; A_{sample} is absorbance of the reaction mixture (DPPH with Sample).

NO RADICAL SCAVENGING ACTIVITY

NO radical scavenging activity of compounds was carried out as per the method of Ramanathan Sambath Kumar *et al*.[22] NO radicals were generated from sodium nitroprusside solution. 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of 1 mM synthetic compounds in phosphate buffer (0.2 M, pH 7.4). The mixture was incubated at 25 °C for 150 min. After incubation the reaction mixture mixed with 1.0 mL of pre-prepared Griess reagent (1 % sulphanilamide, 0.1 % naphthyl ethylenediamine dichloride and 2 % phosphoric acid). The absorbance was measured at 546 nm and the percent inhibition was calculated using the same formula as above. The decreasing absorbance indicates a high nitric oxide scavenging activity.

SUPEROXIDE RADICAL (SOR) SCAVENGING ASSAY

The superoxide anion scavenging activity was performed by the reported method.[25] The reaction mixture consisting of 1 mL of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer, pH 7.4), 1 mL NADH solution (468 mM NADH in phosphate buffer, pH 7.4), and 1 mL of synthetic compound (1 mM) solution was mixed. The reaction was started by adding 1 mL of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm against the blank sample and compared with standard and percentage of inhibition was calculated using the same formula as above. The decreased absorbance of the reaction mixture indicated increased SOR scavenging activity.

HYDROGEN PEROXIDE (H_2O_2) SCAVENGING ACTIVITY

The hydrogen peroxide scavenging assay carried out by the reported method.[26] A solution of hydrogen peroxide (40 mM) prepared in phosphate buffer (pH 7.4). The 1 mM concentrations of various synthetic compounds added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without the drug. The percentage scavenging of hydrogen peroxide by synthetic compounds and standard compounds calculated by using the following formula,

Percentage scavenged (H_2O_2) = $(A_0 - A_1) / A_0 \times 100$

where, A_0 = the absorbance of control; A_1 = the absorbance in presence of the sample of MO and standards.

Anti-inflammatory Activity

IN VITRO ANTI-INFLAMMATORY ACTIVITY BY PROTEIN DENATURATION METHOD

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of synthetic compound (1 mM). A similar volume of double-distilled water served as control. Then the mixtures were incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank. Diclofenac sodium (1 mM) was used as the reference standard and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula,

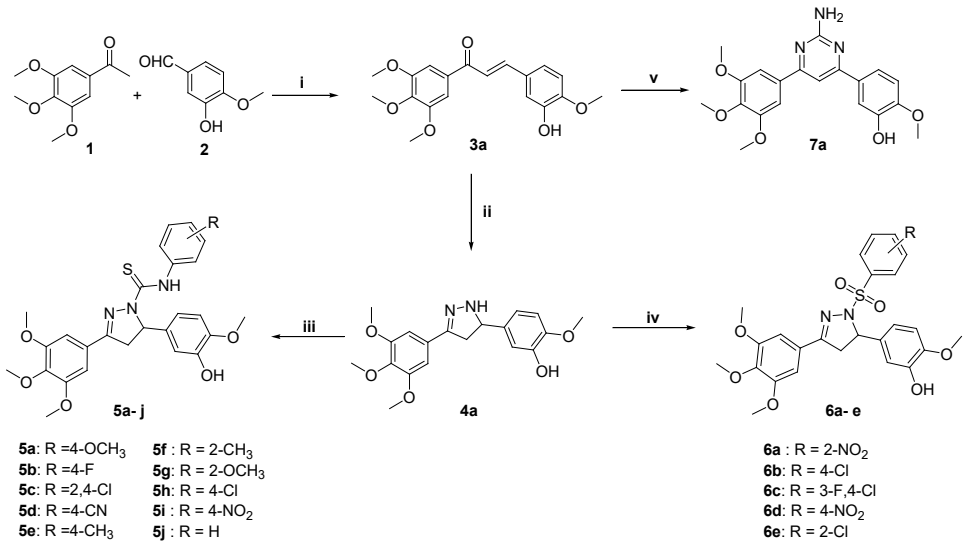
% inhibition = $100 \times (Vt / Vc - 1)$

where, Vt = absorbance of test sample, Vc = absorbance of control.[27]

RESULTS AND DISCUSSION

Chemistry

In the present study, we report three categories of novel analogs of CA-4 having the same substituent on ring A and B with different bridgehead linker, such as 3,5-diaryl-1-carbothioamide-pyrazoline (**5a**–**j**), N[1]-phenyl sulfonyl pyrazoline (**6a**–**e**) and pyrimidine **7a**. The target compounds (**5a**–**j**) and (**6a**–**e**) was accomplished through the reaction between **4a** with differently substituted phenyl isothiocyanate and phenyl sulphonyl chlorides in good yield (**Scheme**



Scheme 1. Reagents and conditions: (i) NaOH, Ethanol, rt, 24 h; (ii) $H_2NNH_2 \cdot H_2O$, Ethanol, 70–80 °C, 16 h; (iii) Phenyl isothiocynate, Ethanol, 70–80 °C, 60 min; (iv) Phenyl Sulphonyl Chloride, Ethanol, 70–80 °C, 1h; (v) guanidine hydrochloride, 10 % NaOH, Ethanol, reflux, 24 h.

1). The starting compound *viz.* pyrazoline analog of CA-4 **4a** for the synthesis of the target compounds was achieved from its precursor chalcone analog of CA-4 **3a** in good yield as per the literature precedent.[12,13] On the other hand, compound **7a** was synthesized by treating compound **3a** with guanidine hydrochloride in the presence of sodium hydroxide *via* 1,4-addition with subsequent rearrangement. The structural investigation of all the synthesized compounds was carried out by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data.

Biological Evaluation

CYTOTOXICITY STUDY

All the synthesized compounds were evaluated for their *in vitro* cytotoxic potencies in human breast cancer cell line MCF-7, besides compound **7a** was also evaluated against human myeloid leukemia cell line K562 using the sulforhodamine B (SRB) assay method. Adriamycin, an effective anticancer drug was used as a reference standard. During the screening process, three response parameters (GI_{50}, TGI, and LC_{50}) were determined. The GI_{50} value (growth inhibitory activity) refers to the concentration of the compound causing 50% reduction in net cell growth, the TGI value (cytostatic activity) fix the concentration of the compound needed for total growth inhibition, and the LC_{50} value (cytotoxic activity) is the concentration of the compound that causes net 50 % loss of initial cells. The calculated response parameters for all the compounds against MCF-7 and for **7a** against K562 are presented in Table 1. Corresponding to the GI_{50} values, a compound's activity is classified as inactive, > 100 μM; moderate, between > 10 and < 100 μM; and active, < 10 μM.

Among the three categories of novel analogs of combretastatin-A4, most of the compounds have shown noticeable cytotoxicity against MCF-7 with the concentration of the drug that produced 50 % inhibition of cell growth (GI_{50}). Compound **7a,** in particular, showed significant cytotoxicity (GI_{50} < 0.1 μM) against the MCF-7 cell line equal to that of adriamycin (GI_{50} <0.1 μM) whereas, compounds **6c**, **5j**, and **5g** also displayed good cytotoxicity (GI_{50} = 25.3–42.6 μM). However, all other compounds showed weak cytotoxicity (GI_{50} = 58.6–100 μM**)** against MCF-7 cell line.

A similar relationship of the TGI concentrations of the compounds in comparison with adriamycin was also carried. Although most of the compounds were inactive, yet compounds **7a** (TGI = 38.58 μM) was found to be most active and compound **5d** (TGI = 86.5 μM) exhibited weak activity against the MCF-7 cell line. All the other compounds were found inactive (TGI > 100 μM) as compared to standard drug adriamycin. Furthermore, the LC_{50} concentrations of the compounds were compared with adriamycin to get an insight into the cytotoxic effects of these compounds against the MCF-7 cell line. All the compounds (LC_{50} >100 μM) like adriamycin (LC_{50} = 97.1 μM) were inactive against the MCF-7 cell line.

Encouraged by the appreciable cytotoxicity exhibited by compound **7a** against MCF-7**,** it was soon after subjected to evaluate cytotoxicity against human myeloid leukemia cell line K562. The results obtained was remarkable with GI_{50} < 0.1 μM, comparable to that of standard drug adriamycin (GI_{50} < 0.1 μM). The TGI concentrations of the compound (TGI >100 μM) was less significant to that of adriamycin (TGI = 75.8 μM). The LC_{50} concentrations of the compound **7a** (LC_{50} > 100 μM) as like adriamycin (LC_{50} > 100 μM) appeared higher against the K562 cell line.

SAR study reveals that (chalcone analog of CA-4) **3a** (GI_{50} < 0.1 μM) with the same substituents on ring A and B was as potent as that of adriamycin, consistent with the IC_{50} = 4.3 nM, and 0.9 μM against K562[11] and MCF-7[28] cell

Table 1. *In vitro* anticancer screening of compounds against MCF-7[(a)] and K562[(a)] cell lines.

Entry	R	MCF-7			K 562		
		LC_{50}[(b)]	TGI[(c)]	GI_{50}[(d)]	LC_{50}[(b)]	TGI[(c)]	GI_{50}[(d)]
3a	–	> 100	> 100	< 0.1	NT	NT	NT
4a	–	> 100	> 100	76.7	NT	NT	NT
5a	4-OCH_3	> 100	> 100	85.9	NT	NT	NT
5b	4-F	> 100	> 100	> 100	NT	NT	NT
5c	2,4-Cl	> 100	> 100	58.6	NT	NT	NT
5d	4-CN	> 100	> 100	87.2	NT	NT	NT
5e	4-CH_3	> 100	> 100	59.1	NT	NT	NT
5f	2-CH_3	> 100	> 100	83.8	NT	NT	NT
5g	2-OCH_3	> 100	> 100	42.6	NT	NT	NT
5h	4-Cl	> 100	> 100	85.9	NT	NT	NT
5i	4-NO_2	> 100	> 100	> 100	NT	NT	NT
5j	H	> 100	99.69	34.75	NT	NT	NT
6a	2-NO_2	> 100	> 100	68.6	NT	NT	NT
6b	4-Cl	> 100	> 100	71.1	NT	NT	NT
6c	3-F, 4-Cl	> 100	86.5	25.3	NT	NT	NT
6d	4-NO_2	> 100	> 100	89.9	NT	NT	NT
6e	2-Cl	> 100	> 100	> 100	NT	NT	NT
7a	–	> 100	38.58	< 0.1	> 100	> 100	< 0.1
Adriamycin	–	> 100	> 100	< 0.1	> 100	75.8	< 0.1

(a) [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.

lines respectively. However, (pyrazoline analog of CA-4) **4a** (GI_{50} = 76.7 μM) showed poor cytotoxicity. An increase in activity was observed when phenyl sulfonyl or phenyl carbothioamide group was substituted at N^1-position of pyrazoline ring. Compound **6c, 5j**, **5g, 5c, 5e, 6a**, and **6b** showed better cytotoxicity than that of **4a.** Furthermore, pyrimidine analog of CA-4 **7a** displayed significant cytotoxicity against both K562 and MCF-7 cell line. From this evidences, a general specific trend in structure and activity cannot be established. Since, chalcone and pyrazoline analog of CA-4 adopt twisted geometry[11,12] like that of CA-4, which is indispensable to fit into the binding site of tubulin to inhibit tubulin polymerization. However, among the synthesized pyrazoline analog (**5a–j**) and (**6a–g**), none of the compounds was as a potent as that of CA-4. On the contrary, the pyrimidine analog **7a** being coplanar, established from the available characterization data, could act by a different mechanism to disclose its cytotoxicity, since a small change in the structure of CA-4 analog has shown the surprising effect on other biological targets.[29]

ANTI-INFLAMMATORY ACTIVITY

Denaturation of proteins is a well- known recognized basis of inflammation. In this study, all the synthesized compounds were evaluated for *in vitro* anti-inflammatory activity by protein denaturation of egg albumin method and results are presented in Table 2. Compound **6a** and **6d** showed good inhibition (81.65–79.81 %) compared to the diclofenac sodium, a standard anti-inflammation drug (90.21 %) at 1mM concentration. Compounds **6b, 5b, 6c**, and **6e** showed effective inhibition of heat-induced albumin denaturation (76.14–72.47 %). However, rest of the compounds showed moderate inhibition.

ANTIOXIDANT ACTIVITY

Overproduction of reactive oxygen species (ROS) contributes to pathophysiology associated with various inflammatory disorders.[30] These radicals can cause damage to cell components such as proteins, lipids, sugars and nucleotides,[13] and may compel the cell from performing its normal physiological functions together with induction of oxidative stress. Antioxidants are the compounds capable of scavenging the free radicals, an option to combat against excessively generated free radicals. Therefore, all the synthesized compound **3a, 4a,** (**5a–j**), (**6a–e)** and **7a** were evaluated against a variety of reactive oxygen and nitrogen species such as 2,2-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), superoxide (SOR) and hydrogen peroxide (H_2O_2). Free radical scavenging activity was determined as percent inhibition and results are summarized in Table 2. All the synthesized compounds have shown good to excellent scavenging activity against DPPH, NO and SOR radicals.

Among the series, compound **6c, 6d,** and **6e** (56.66–61.11 %) were excellent inhibitors of DPPH radical, compared to standard drug ascorbic acid (44.18 %) whereas compound **5d** showed (43.33 %) moderate inhibition of DPPH radical. However, rests of the compounds were devoid of activity.

In case of NO radical scavenging activity, compounds **3a, 4a, 5f, 6a, 6b, 6c, 6d**, and **6e** showed excellent activity (46.66–61.66 %) as compared to standard drug ascorbic acid (42.63 %). Besides, compounds **5e** and **5a** exhibited moderate activity (41.80–34.42 %) whereas, the remaining compounds were inactive.

The SOR scavenging activity results revealed that most of the synthesized compound displayed remarkable activity except, compound **5**i and **6e** (35.71–39.28 %). Compounds **3a, 4a, 5a–h, 5j**, and **6a–d** found to possess excellent SOR scavenging activity (78.57–92.85 %) compared to a standard drug ascorbic acid (74.07 %).

Table 2. Anti-inflammatory and antioxidant activities of synthesized compounds.

Entry	R	Anti-inflammatory	Antioxidant activity			
		% inhibition (1 mM)				
		Egg albumin	DPPH	NO	SOR	H_2O_2
3a	–	64.22	23.33	51.66	85.71	28.82
4a	–	69.72	30.00	46.66	91.07	44.49
5a	4-OCH_3	66.97	21.11	40.00	92.85	29.37
5b	4-F	74.31	23.33	31.66	87.50	17.67
5c	2,4-Cl	49.54	15.55	08.33	91.07	06.65
5d	4-CN	58.71	43.33	5.00	89.28	22.96
5e	4-CH_3	63.30	23.33	41.66	92.85	41.72
5f	2-CH_3	72.47	20.00	50.00	92.85	42.38
5g	2-OCH_3	69.72	11.11	25.00	91.07	28.25
5h	4-Cl	61.46	23.33	33.33	85.71	26.21
5i	4-NO_2	55.96	05.00	05.00	35.71	13.76
5j	H	61.46	21.11	23.33	78.57	40.33
6a	2-NO_2	81.65	34.44	61.66	85.71	32.61
6b	4-Cl	76.14	30.00	55.00	82.14	43.02
6c	3-F, 4-Cl	74.31	56.66	56.66	83.92	38.95
6d	4-NO_2	79.81	53.33	56.66	89.28	36.51
6e	2-Cl	72.47	61.11	53.33	38.28	37.59
7a	–	64.22	15.55	61.66	91.07	37.80
Control	–	–	–	–	–	–
AA	–	–	44.18	42.63	74.07	47.17
DS	–	90.21	–	–	–	–

ND = Not Determined; AA = Ascorbic acid; DS = Diclofenac Sodium.

On the contrary, all compounds evaluated against hydrogen peroxide demonstrated well to moderate activity. Compounds **4a, 5e, 5f, 5j,** and **6b** (40.33–44.49 %) showed good activity as compared to reference standard ascorbic acid (47.17 %) whereas, compounds **6c, 6d**, and **6e** (36.55–38.95 %) exhibited moderate activity and all other compounds were poor inhibitors of H_2O_2 radical.

CONCLUSION

In conclusion, we have synthesized a diverse library of pyrazole and pyrimidine analogs of CA-4 and evaluated *in vitro* as potential antitumor, anti-inflammatory, and antioxidant agents. Results of the anticancer screening disclosed compound **7a,** a potential lead candidate that possess potent anti-proliferative activity against MCF-7 and K562, with GI_{50} inhibitory values <0.1 μM respectively. Compounds **6c**, **5j** and **5g** also displayed good cytotoxicity against MCF-7 (GI_{50} = 25.3–42.6 μM). On the other hand, compound **6a** and **6d** showed good inhibition of protein denaturation (81.65–79.81 %) compared to the standard drug diclofenac sodium (90.21 %). However, most of the compounds screened were found to procure good to excellent DPPH, NO and SOR radical scavenging activity.

Acknowledgment. We are very thankful to the School of Chemical Sciences, Solapur University, Solapur for providing laboratory facilities and also offer thanks to ACTREC Mumbai for providing anticancer activity determinations. We also thank Instrumentation Centre, Solapur University for providing facility of spectral analysis.

REFERENCES

[1] M. Malumbres, M. Barbacid, *Nat. Rev. Cancer.* **2001**, *1*, 222.

[2] L. Ying-Rui, L. Ji-Zhuang, D. Pan-Pan, Z. Bao-Xiang, M. Jun-Ying, *Bioorg Med. Chem. Lett.* **2012**, *22*, 6882.

[3] O. Valiron, N. Caudron, D. Job, *Cell. Mol. Life Sci*. **2001**, *58*, 2069.

[4] G. R. Pettit, S. B. Singh, M. L. Niven, E. Hamel, J. M. Schmidt, *J. Nat. Prod*. **1987**, *50*, 119.

[5] C. M. Lin, S. B. Singh, P. S. Chu, R. O. Dempcy, J. M. Schmidt, G. R. Pettit, E. Hame, *Mol. Pharmacol*. **1988**, *34*, 200.

[6] C. M. Lin, H. H. Ho, G. R. Pettit, E. Hamel, *Biochemistry* **1989**, *28*, 6984.

[7] D. J. Chaplin, G. R. Pettit, S. A. Hill, *Anticancer Res.* **1999**, *19*, 189.

[8] (a) 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; (b) G. M. Tozer, C. Kanthou, C. S. Parkins, S. A. Hill, *Int. J. Exp. Pathol*. **2002**, *83*, 21.

[9] K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama, T. Tsuji, *J. Med. Chem*. **1998**, *41*, 3022.

[10] J. A. Woods, J. A. Hadfield, G. R. Pettit, B. W. Fox, A. T. McGown, *Br. J. Cancer*. **1995**, *71*, 705.

[11] J-P. Liou, Y-L. Chang, F-M. Kuo, C-W. Chang, H. Y. Tseng, C-C. Wang, Y-N Yang, J-Y. Chang, S-J. Lee, H-P. Hsieh, *J. Med. Chem*. **2004**, *47*, 4247.

[12] S. Ducki, R. Forrest, J. A. Hadfield, A. Kendall, N. J. Lawrence, A. T. McGown, D. Rennison, *Bioorg. Med. Chem. Lett*. **1998**, *8,* 1051.

[13] M. Johnson, B. Younglove, L. Lee, R. LeBlanc, H. Holt Jr., P. Hills, H. Mackay, T. Brown, S. L. Mooberry, M. Lee, *Bioorg. Med. Chem. Lett*. **2007**, *17*, 5897.

[14] A. P. Keche, G. D. Hatnapure, R. H. Tale, A. H. Rodge, V. M. Kamble, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6611.

[15] R. Fioravanti, A. Bolasco, F. Manna, F. Rossi, F. Orallo, F. Ortuso, S. Alcaro, R. Cirilli, *Eur. J. Med. Chem*. **2010**, *45*, 6135.

[16] C. Congiu, V. Onnis, L. Vescil, M. Castorina, C. Pisano, *Bioorg. Med. Chem*. **2010**, *18*, 6238.

[17] N. Gokhan-Kelekci, S. Yabanoglu, E. Kupeli, U. Salgin, O. Ozgen, G. Ucar, E. Yesilada, E. Kendi, A. Yesilade, A. Bilgin, *Bioorg. Med. Chem*. **2007**, *15*, 5775.

[18] T-S Jeong, K-S Kim, J-R Kim, K-H Cho, S. Lee, W. Lee, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2697.

[19] H. Jiqiang, M. Liang, W. Zhe, Z. Jun, P. Fei, S. Mingfeng, L. Lei, H. Lin, T. Minghai, H. Linhong, W. Yuzhe, C. Lijuan, *Bioorg. Med. Chem. Lett*. **2015**, *25*, 2429.

[20] (a) S. Shenvi, K. Kumar, K. S. Hatti, K. Rijesh, L. Diwakar, G. C. Reddy, *Eur. J. Med. Chem*. **2013**, *62*, 435; (b) H. S. Kareem, A. Ariffin, N. Nordin, T. Heidelberg, A. Abdul-Aziz, K.W. Kong, W.A. Yehye, *Eur. J. Med. Chem*. **2015**, *103*, 497.

[21] (a) B. P. Bandgar, S. S. Jalde, B. L. Korbad, S. A. Patil, H. V. Chavan, S. N. Kinkar, L. K. Adsul, S. N. Shringare, S. H. Nile, *J. Enzyme Inhib. Med. Chem*. **2012**, *27*, 267; (b) B. P. Bandgar, L. K. Adsul, H. V. Chavan, S. S. Jalde, S. N. Shringare, R. Shaikh, R. J. Meshram, R. N. Gacche, V. Masand, *Bioorg. Med. Chem. Lett*. **2012**, *22*, 5839; (c) B. P. Bandgar, L. K. Adsul, S. V. Lonikar, H. V. Chavan, S. N. Shringare, S. A. Patil, S. S. Jalde, B. A. Koti, N. A. Dhole, R. N. Gacche, A. Shrifule, *J. Enzyme Inhib.Med. Chem*. **2013**, *28*, 593; (d) B. P. Bandgar, R. J. Sarangdhar, F. Khan, J. Mookkan, S. Choudhary, H. V. Chavan, S. B. Bandgar, V. Y. Kshirsagar, *Eur. J. Med. Chem.* **2012**, *57*, 217; (e) H. V. Chavan, L. K. Adsul, A. S. Kotmale, V. D. Dhakane, V. N. Thakare, B. P. Bandgar, *J. Enzyme Inhib.Med. Chem*. **2015**, *30*, 22.

[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.

[23] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. Mcmohan, D. Vistica, T. Warren, H. Bokesch, S. Kenney, M. Boyd, *J. Nat. Cancer Inst.* **1990**, *82*, 1107.

[24] R. S. Kumar, T. Sivakumar, R. S. Sundaram, P. Sivakumar, R. Nethaji, M. Gupta, U. K. Mazumdar, *Iranian. J. Pharmacol. Therap*. **2006**, *5*, 35.

[25] G. I. Ilhami, A. A. Haci, C. Mehmet, *Chem. Pharm. Bull.* **2005**, *53*, 281.

[26] J. Robak, R. J. Gryglewski, *Biochem. Pharmacol.* **1988**, *37*, 837.

[27] Y. Mizushima, M. Kobayash, *J. Pharma. Pharmacol*. **1968**, *20*, 169.

[28] Y. Kong, K. Wang, M. Edler, E. Hamel, S. L. Mooberry, M. Paige, M. L. Brown, *Bioorg. Med. Chem.* **2010**, *18*, 971.

[29] (a) C. Cox, M. Breslin, B. Mariano, P. Coleman, C. Buser, E. Walsh, K. Hamilton, H. Huber, N. Kohl, M. Torrent, Y. Yan, L. Kuo, Hartman, *Bioorg. Med. Chem. Lett.* **2011**, *15*, 2041; (b) J. T. Gupton, B. S. Burham, K. Krumpe, K. Du, J. A. Sikorski, A. E. Warren, C. R. Barnes, I. H. Hall, *Arch. Pharm.* **2000**, *333*, 3.

[30] A. A. Geronikaki, A. M. Gavalas, *Comb. Chem. High Throughput Screen.* **2006**, *9*, 425.