Research Article

Synthesis and Antiproliferative Activity of Novel ١,٢,٤-Triazoles as potential combretastatin analogues.

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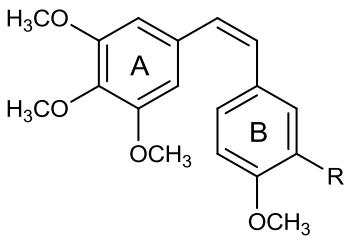
Abstract
Combretastatin A٤ (CA٤) is a simple natural compound discovered three decades ago, it's characterized by a very powerful inhibition of tubulin polymerization. However, CA٤ was less active in vivo because the cisoid configuration is transformed into trans. In our work we designed and synthesized new *cis* restricted combretastatin analogues by introducing a heterocyclic five membered ring "١,٢,٤-triazle" instead of the carbon-carbon double bond.
Key Words: Combretastatin, Synthesis, Antiproliferative

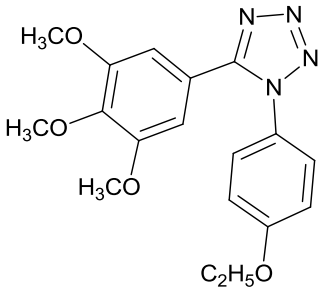
Introduction
Combretastatin A٤ (CA٤) is a simple natural product discovered three decades ago. It was isolated from the stem wood of the South African tree *Combretum caffrum* [١]. CA٤ showed structural similarity with most tubulin polymerization inhibitors resulting in higher matching with colchicine binding site of tubulin [٢]. It was found that CA٤ binds to tubulin dimers and preventing microtubule polymerization leading them to apoptosis [٣], CA٤ is inactive *in vivo* because of its lower solubility in water, this problem was solved by synthesis of more soluble prodrugs, phosphate disodium (CA-٤P) and a serinamido derivative (AVE-٨٠٦٢) that showed promising results in clinical trials on the anaplastic thyroid carcinoma.[٤,٥] SAR study of CA-٤ has shown that the trimethoxy group in ring A, the cisoid configuration at the bridge and presence of methoxy group in *para* position on ring B are all critical for the cytotoxic potency [٦]. It was revealed that the *cis*. olifenic double bond is transformed *in vivo* into *trans* isomer due to rotation of ring A and B around the ethylene bridge leading to great loss in activity [٧].

Therefore, several studies were done to prevent this rotation, this was accomplished by introducing hetero-aromatic rings such as isomeric triazoles [٨], tetrazole [٩] to rigidify the structure. In our work, we introduced ١,٢,٤-triazole ring instead of the carbon-carbon double bond of CA-٤ using ٣,٤,٥-trimethoxy phenyl moiety as ring A and ٣,٤-difluorophenyl moiety as ring B. Moreovere, we introduced a third ring (ring C) searching for extra-binding with the colchicine binding site of tubulin. These synthesized compounds were tested for their cytotoxicity by the (NCI).



Z-1,2-diarylethylene

CA-4 R = OH
CA-4P R = OPO_3Na_2
AVE-8062 R = NH-ser, HCL



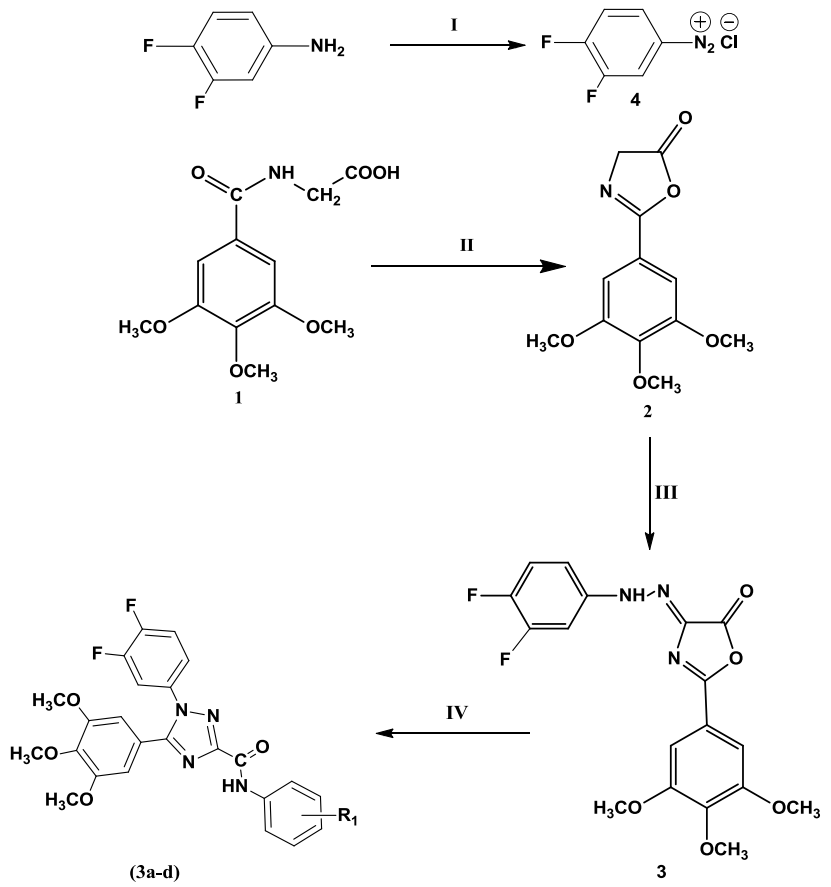
1,5-disubstituted tetrazole

Figure ١: Examples combretastatin A٤ and its analogues.

Results and discussions

Chemistry

The targeted compounds of triazole carboxylic acid derivatives were synthesized upon the basis of scheme١. Treating of ٣,٤,٥-trimethoxy benzoyl chloride with glycine in ١٠٪ NaOH provided the starting compound ٢-(٣,٤,٥-trimethoxy benzamido) acetic acid ١ in a good yield ٨٥٪ . Compound ١ was heated at ٦٠°C for ٥٠ min to afford an oxazoline-٢-one derivative ٢. Using kuskuv like reaction; compounds ٣ was prepared by coupling the diazonium salt of ٣,٤-difluoro aniline with the active methylene group of ٢ in acetic acid in presence of anhydrous sodium acetate at ٠-٥°C. Based on Sawdey rearrangement; [١٠] treating compound ٣ with primary amine in acidic media will rearrange to afford the targeted compounds **(٣a-d)**.



٣a: R١= ٢-OCH٣; ٣b: R١=٤-Cl; ٣c: R١= ٢,٣-F; ٣d: R١=٤-CH٣

Scheme ١ (series ١&٢): (I) NaNO٢, HCl, -٥-٠°C; (II) AC٢O, ٦٠°C, ٥٠min; (III) ٣,٤-difluoro-benzenediazonium chloride ٤, NaOAC, AcOH, ٠-٥°C; (IV) aromatic amine, NaOAc, AcOH, Reflux, ٢h.

Antiproliferative investigation against ٦٠ cell lines at the NCI

The synthesized compounds were submitted to National Cancer Institute (NCI) at www.dtp.nci.nih.gov. The compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of ٦٠ cell lines derived from nine different cancer types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers). The results showed that compound **٣c** exhibited the highest percentages of inhibition against most cell lines. A significant cell growth inhibition was observed against lung cancer HOP-٩٢, and CNS cancer SNB-٧٥, cell lines. (Table

١) Moreover, compound **٣c** exhibited moderate cytotoxic activity against leukemia MOLT-٤, RPMI-٨٢٢٦, SR, lung cancer A٥٤٩/ATCC, EKVX, HOP-٦٢, NCI-H٢٢٦, NCI-H٥٢٢, colon cancer HCT-١٥, melanoma SK-MEL-٥, UACC-٦٢, ovarian cancer IGROV١, OVCAR-٤, SK-OV-٣, renal cancer A٤٩٨, RXF ٣٩٣, UO-٣١, prostate cancer PC-٣, and breast cancer MCF٧, MDA-MB-٢٣١/ATCC, HS ٥٧٨T, T-٤٧D cell lines. Furthermore, compound **٣c** revealed remarkable activity against leukemia CCRF-CEM, HL-٦٠, K-٥٦٢, lung cancer NCI-H٢٣, NCI-H٣٢٢M, NCI-H٤٦٠, colon cancer COLO ٢٠٥, HCT-١١٦, HT٢٩, CNS cancer SF-٢٦٨, SF-٢٩٥, SF-٥٣٩, SNB-١٩, U٢٥١, melanoma LOX IMVI, MALME-٣M, M١٤, SK-MEL-٢, ovarian cancer OVCAR-٣, OVCAR-٨, NCI/ADR-RES, renal cancer ٧٨٦-٠, ACHN, SN١٢C, and breast cancer MB-٤٦٨ cell lines.

Compound **٣a** exhibited moderate activity against leukemia K-٥٦٢, SR, lung cancer HOP-٩٢, NCI-H٥٢٢, colon cancer HCT-١٥, CNS cancer SNB-٧٥, melanoma MDA-MB-٤٣٥, SK-MEL-٥, UACC-٦٢, renal cancer A٤٩٨, UO-٣١, prostate cancer PC-٣, and breast cancer MDA-MB-٢٣١/ATCC, T-٤٧D cell lines. On the other hand, compound **٣b** exhibited moderate cell growth inhibition activity against leukemia RPMI-٨٢٢٦, SR, lung cancer NCI-H٥٢٢, CNS cancer SNB-٧٥, renal cancer A٤٩٨, UO-٣١, prostate cancer PC-٣, and breast cancer MDA-MB-٢٣١/ATCC, T-٤٧D cell lines. While compound **٣d** showed decreased activity agains most tested cell lines.

The results reported, revealed that compound **٣c** that bears two fluorine atoms on ring C showed the highest growth inhibition percentages; this may be attributed to the formation of non-polarizable C-F bond resulting from the compatible overlap between ٢S and ٢P orbitals, as a result of this property, the lipophilicity will be enhanced.

Experimental

Chemistry

Reactions were monitored by thin layer chromatography (TLC), using Merck ٩٣٨٥ pre-coated aluminum plate silica gel (Kieselgel ٦٠) ٥ cm _٢٠cm plates with a layer thickness of ٠.٢ mm. The spots were detected by exposure to UV-lamp at ٢٥٤ nm. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. NMR spectra were carried out using a Bruker Avance ٣٠٠MHz NMR spectrometer, using TMS as internal reference. Chemical shifts (d values are given in parts per million (ppm) relative to $CDC^{١٣}$ (٧.٢٩ for proton and ٧٦.٩ for carbon) and coupling constants (J) in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. Elemental analysis was performed on Vario El Elementar CHN Elemental analyzer; organic microanalysis section, Cairo University, Giza, Egypt and the results were within ٠.٤٪ of the theoretical values.

Table (١): % of cell growth inhibition against different cancer cell lines.

Panel/Cell Line	% Growth Inhibition				Panel/Cell Line	% Growth Inhibition			
	٣a	٣b	٣c	٣d		٣a	٣b	٣c	٣d
Leukemia					**Melanoma**				
CCRF-CEM	٩.٤٩	٣.٠٤	٢١.٠٦	٠	LOX IMVI	٤.٦٣	٢.٥٦	١٥.١٤	١.٠٢
HL-٦٠(TB)	٢٧.٦١	٧.٤٥	٢٤.٢٢	٠	MALME-٣M	٠	٥.٨٦	١٥.٣٧	١٠.٩٩
K-٥٦٢	٣٦.١٤	٧.٦٥	١٨.٦٦	٠	M١٤	١٠.٧١	١٢.٠٢	١٩.٥٦	٥.٣٦
MOLT-٤	٢٠.٣٤	٢٤.٢٩	٣٢.٢٦	٥.١٢	MDA-MB-٤٣٥	٥٦.٨١	١١.٥٣	١٢.٠٩	٦.٧٤
RPMI-٨٢٢٦	٢٧.٧٦	٣٠.٩٦	٣٥.٦٣	٠	SK-MEL-٢	٢٢.٨١	٨.٣٩	٢٠.٩٨	١٠.٤٥
SR	٦٠.٠١	٢٩.٢٧	٣٦.٠٣	٨.١٧	SK-MEL-٢٨	١٢.٩٢	٣.٥٨	٩.٥٨	٠
Non-Small Cell Lung Cancer					SK-MEL-٥	٣٢.٩٦	٢٣.٤٣	٣٧.٨٥	٤.١٧
A٥٤٩/ATCC	٢٤.٦٦	١١.٣٢	٣٠.٢١	٣.٧٩	UACC-٢٥٧	٦.٨٤	١٠.٨٨	٧.٧٨	N.D.
EKVX	١٩.٩٣	٨.٥٢	٣٢.٤١	٠.١٣	UACC-٦٢	٣٣.١٢	١٨.٢٦	٣٧.١٢	٠
HOP-٦٢	٢٢.٨٥	٧.٧٨	٣١.٧٨	١٤	**Ovarian Cancer**				
HOP-٩٢	٥٠.٢	١٨.٥١	٧٧.٥٣	٠	IGROV١	٧.٨٥	١.١٥	٣١.٨٤	١٣.٠٧
NCI-H٢٢٦	١٩.٩	١٢.٦	٢٩.٢٣	٠	OVCAR-٣	٠.٤	٠	١٣.٩٣	٠
NCI-H٢٣	٨.٠٤	٧.٤٥	٢٠.٣٤	٢.٨٧	OVCAR-٤	٢٦.٣٨	٢٠.٢٣	٤٢.٥٤	٠
NCI-H٣٢٢M	١.٧٩	٦.٦٧	١٦.٣٢	٤.٠١	OVCAR-٥	٠.٧٨	٠	٧.٠٢	٠
NCI-H٤٦٠	٦.٠٨	٤.٣٩	١٣.٣٢	٠	OVCAR-٨	١٤.١٣	٦.١٨	١٧.٣٥	٠
NCI-H٥٢٢	٤٧.٧٨	٣٧.١٣	٤٧.٣٨	٥١.٩٦	NCI/ADR-RES	١٥.٩٢	٧.٤٣	٢٨.٢٦	٦.٣٨
Colon Cancer					SK-OV-٣	٢٤.٢٩	١٧.٢٥	٤٦.٢٩	١٠.٩٢
COLO ٢٠٥	٩.٩١	٠	٢٣.٧	٤.١٨	**Renal Cancer**				
HCC-٢٩٩٨	٠	٠	٨.٩	١٣.٤	٧٨٦-٠	١٨.١٤	٢٠.٦٩	٢١.٩٧	١.٤
HCT-١١٦	٢٠.٧٩	٢١.٥٤	٢٧.٠٤	٤.٣١	A٤٩٨	٣٦.١٧	٤٢.٢٨	٥٨.٣٧	٠
HCT-١٥	٣٥.٩	١٢.٢١	٣٧.٦٤	٥.٢٨	ACHN	١٤.٥٢	٦.٤٤	٢٨.٧٩	٠
HT٢٩	١٩.٤	١٠.٩٧	١٧.٧٨	٢٤.٥٢	CAKI-١	N.D	N.D.	N.D.	N.D.
KM١٢	١٨.٨٥	١.١٢	١٢.٨٥	٠	RXF ٣٩٣	٨.٠٧	١٥.٩٣	٢٩.١٨	٠
SW-٦٢٠	٤.٤٥	٠	٥.٣	٠	SN١٢C	١٦.٧٩	٢١.٢٥	٢٦.٣٧	٢.٥٣
CNS cancer					TK-١٠	٥.٤٢	٦.٣٨	٥.٤٦	١.١٧
SF-٢٦٨	٧.٦٤	١٩.٨٥	٢٦.٨٨	٢٦.٨٨	UO-٣١	٣٩.٥٦	٣٩.٣٩	٦٢.٤١	٢٧.٠٦
SF-٢٩٥	١٤.٠٧	٥.٩٨	١٤.١٨	١٤.١٨	**Breast Cancer**				
SF-٥٣٩	٧.٦٤	٣.٤٤	١٩.٣٢	١٩.٣٢	MCF٧	٢٤.٥٥	١٤.٧٣	٤٠.٨١	٤.٩٣
SNB-١٩	١٨.٤٣	١٣.١٦	٢٦.٥٥	٢٦.٥٥	MDA-MB٢٣١/ATCC	٣٣.٦٨	٢٩.٢٦	٤٧.٠٥	٣.٨٨
SNB-٧٥	٣١.٩	٤٦.٣١	٦٨.٥٨	٦٨.٥٨	HS ٥٧٨T	١٨.٥١	٢٣	٣٥.٦٤	٠
U٢٥١	١٦.٥٢	٧.٦١	٢٥.٩٢	٢٥.٩٢	BT-٥٤٩	١٦.٤١	٢٠.٣٨	١٨.١٥	٠
Prostate Cancer					T-٤٧D	٤٢.٣٥	٣٨.٣٥	٥٥.٨٥	٢٢.٩
PC-٣	٣٨.١٦	٣٠.٧١	٥٥.٩٦	٣.٢٨	MDA-MB-٤٦٨	١٢.٥٢	٠	٢٨.٨١	٠
DU-١٤٥	٠	٠	٠.٧٧	٠					

٣.١.١. Synthesis of ٤-[(٤-Ethoxy-phenyl)-hydrazono]-٢-(٣,٤,٥-trimethoxy-phenyl)-٤*H*-oxazol-٥-one **٣**

Trimethoxyhippuric acid **١** (٠.١٧ mol, ٤٦.٦٧ g)) was heated with acetic anhydride (١٠٠ ml) at ٦٠°C for ٥٠ min or until a clear

solution of ٢ was obtained; solution will be cooled to room temperature (solution A). Stirring ٣,٤-difluoro aniline (٠.١٣ mol, ٢٣.٣٢ g) with ٥N HCl (٤٠ mL) and glacial acetic acid (٤٠ mL) in an ice-salt bath ⁻٥ to ٠°C, a solution of sodium nitrite (٠.١٧ mol, ١١.٩٦ g) in water (٢٠ ml) was added in a drop wise manner. The reaction mixture was left for ١٠ min then anhydrous sodium acetate (٠.٢٤ mol, ٢٠ g) was added (solution B). Solution A was added to solution B in a drop wise manner at ٠–١٠°C and stirring for ٢h; the formed precipitate was filtered off and dried to afford light red solid (٢.٠٠g, ٦٧٪ yield).

٣.١.٢. General procedure for the synthesis of ١-(٣,٤-difluorophenyl)- ٥-(٣,٤,٥-trimethoxy-phenyl)-١*H*-١,٢,٤-triazole-٣-carboxamides **(٣a-d)**.

A mixture of compound ٣ (٣.٩٩ g, ٠.٠١ mol) and appropriate primary aromatic amine (٠.٠١ mol) was refluxed in acetic acid (٥٠ ml) in the presence of anhydrous sodium acetate (١.٥ g, ٠.٠١٨ mol) for ٢ h.

The reaction mixture was cooled and poured into ice water (٥٠ ml) while stirring. The formed precipitate was filtered off, dried, and recrystallized from methanol.

٣.١.٣. ١-(٣,٤-Difluoro-phenyl)-٥-(٣,٤,٥-trimethoxy-phenyl)-١H-[١,٢,٤]triazole-٣-carboxylic acid (٢-methoxy-phenyl)-amide **٣a**

Yellowish brown crystals (٣.٢١ g, ٦٤٪ yield); m.p. ١٧٨-١٨١°C; ^{١}HNMR (٤٠٠MHz, $CDCl_{٣}$) δ (ppm): ٣.٧٧(s, ٦H), ٣.٩٢(s, ٣H), ٣.٩٧(s, ٣H), ٦.٧٨(s, ٢H), ٦.٩٦(d, ١H, *J* =٨.٠١ Hz), ٧.٠٦(t, ١H, *J* =٧.٦٥ Hz), ٧.١٤(t, ١H, *J* =٧.٦٣ Hz), ٧.٢٤-٧.٣٢(m, ٢H), ٧.٤٤(t, ١H, *J* =٧.٣٣ Hz), ٨.٦٢(d, ١H, *J* =٧.٨٩ Hz), ٩.٦٠(s, ١H); ^{١٣}CNMR(١٠٠ MHz, $CDCl_{٣}$, δppm): ٥٥.٨٦, ٥٦.٢٣, ٦١.٠٣, ١٠٦.٥٩, ١١٠.٠٦, ١١٥.٥٥, ١١٧.٨٨, ١١٨.٠٧, ١٢٠.٣٠, ١٢١.٤٤, ١٢٢.١٦, ١٢٤.٤٤, ١٢٧.١١, ١٣٣.٦٤, ١٣٤.٠٥, ١٤٠.٤٠, ١٤٨.٣٥, ١٤٩.٠١, ١٤٩.٥٠, ١٥١.٣٩, ١٥٢.٠٢, ١٥٣.٤٣, ١٥٥.١٧, ١٥٦.٣٠, ١٥٦.٩٩.

١-(٣,٤-Difluoro-phenyl)-٥-(٣,٤,٥-trimethoxy-phenyl)-١H-[١,٢,٤]triazole-٣-carboxylic acid (٤-chloro-phenyl)-amide **٣b**

Brown crystals (٢.٩١ g, ٥٨% yield); m.p. ١٥٧-١٥٩°C; ^{١}HNMR (٤٠٠MHz, $CDCl_{٣}$) δ (ppm): ٣.٧٧(s, ٦H), ٣.٩٣(s, ٣H), ٦.٧٥(s, ٢H), ٧.٢٤-٧.٣٢(m, ٣H), ٧.٣٨(d, ٢H, *J* =٨.٧٢ Hz), ٧.٧٥(d, ٢H, *J* =٨.٧٢ Hz), ٩.٠٢(s, ١H); ^{١٣}CNMR(١٠٠ MHz, $CDCl_{٣}$, δppm): ٥٦.٢٥, ٦١.٠٥, ١٠٦.٤٧, ١١٥.٤٨, ١١٥.٦٩, ١١٨.١٧, ١٢١.٠٩, ١٢١.١٥, ١٢٢.١٠, ١٢٩.٠٠, ١٢٩.٢٣, ١٢٩.٨٨, ١٣٣.٨٣, ١٣٥.٨٥, ١٤٠.٥٦, ١٤٩.٤٦, ١٥٣.٥٢, ١٥٥.٢٣, ١٥٦.٣٥.

٣.١.٤. N-(٢,٣-difluorophenyl)-١-(٣,٤-difluorophenyl)-٥-(٣,٤,٥-trimethoxyphenyl)-١H-١,٢,٤-triazole-٣-carboxamide **٣c**

Yellow crystals (٣.٢٢ g, ٦٣٪ yield); m.p. ١٦٧-١٦٩°C; ^{١}HNMR (٤٠٠MHz, $CDCl_{٣}$) δ (ppm): ٣.٧٧(s, ٦H), ٣.٩٢(s, ٣H), ٦.٧٧(s, ٢H), ٦.٩٦-٧.٠٢(m, ١H), ٧.١٦(d, ١H, *J* =٥.٩٥ Hz), ٧.٢٥-٧.٣٣(m, ٢H), ٧.٤٣(t, ١H, *J* =٨.٤١ Hz), ٨.٣٤(d, ١H, *J* =٧.٤٥ Hz), ٩.٢٧(s, ١H); ^{١٣}CNMR(١٠٠ MHz, $CDCl_{٣}$, δppm): ٥٦.١٦, ٥٦.٤١, ٦١.٠٣, ١٠٦.٤٣, ١١٢.٦٣, ١١٥.٧٦, ١١٦.٧٧, ١١٨.٠٠, ١٢١.٠٣, ١٢٢.١٦, ١٢٤.٣٧, ١٣٣.٨٤, ١٤٠.٥٥, ١٤٢.٦١, ١٤٨.٩١, ١٤٩.٠٥, ١٥١.٤٧, ١٥٢.١٥, ١٥٣.٤٨, ١٥٥٤١, ١٥٦.١٠, ١٥٦.٤٨.

٣.١.٥. ١-(٣,٤-difluorophenyl)-N-(p-tolyl)-٥-(٣,٤,٥-trimethoxyphenyl)-١H-١,٢,٤-triazole-٣-carboxamide **٣d**

Gray crystals (٣.١٠ g, ٦٥٪ yield); m.p. ١٥٤-١٥٦°C; ^{١}HNMR (٤٠٠MHz, $CDCl_{٣}$) δ (ppm): ٢.٣٨(s, ٣H), ٣.٧٨(s, ٦H), ٣.٩٢(s, ٣H), ٦.٧٦(s, ٢H), ٧.٢٢(d, ٢H, *J* =٨.٣٢ Hz), ٧.٢٧-٧.٣٢(m, ٢H), ٧.٤٣(t, ١H, *J* =٩.٦١ Hz), ٧.٦٧(d, ٢H, *J* =٨.٣٣ Hz), ٨.٩٩(s, ١H); ^{١٣}CNMR(١٠٠ MHz, $CDCl_{٣}$, δppm): ٢٠.٩٦, ٥٦.٢١, ٦١.٠٤, ١٠٦.٤٧, ١١٥.٤٩, ١١٥.٦٩, ١١٧.٩٤, ١١٨.١٢, ١١٩.٩٤, ١٢١.١٧, ١٢٢.٠٧, ١٢٩.٦٦, ١٣٤.٥٤, ١٣٤.٧٥, ١٤٠.٥٠, ١٤٩.٤١, ١٥٣.٥٠, ١٥٥.٠٦, ١٥٦.١٦, ١٥٦.٦٤.

٣.٢. Biology

٣.٢.١ Sixty cancer cell line screening at the NCI

The methodology of the NCI anticancer screening has been described in detail at (http://www.dtp.nci.nih.gov). Briefly, the anticancer assay was performed at approximately ٦٠ human tumor cell lines panel derived from nine cancer cells, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. The tested anilides were added to the culture at a single concentration ($١٠^{-٥}$ M) and the cultures were incubated for ٤٨ h. End point determinations were made with a protein

binding dye, SRB. Results for each tested anilide were reported as the percent of tumor growth of the treated cells in comparison with the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

Conclusion

Novel ١,٢,٤-triazole-٣-carboxylic acid derivatives were synthesized resembling *cis* restricted combretastatin analogues **(٣a-d)**, these compounds showed moderate to good anticancer activities and their structures were emphasized by different spectroscopic techniques. The results reported, revealed that compound **٣c** that bears two fluorine atoms on ring C showed the highest growth inhibition percentages in NCI٦٠ assay may be because of the enhanced lipophilicity.

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