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ATRA-hydrazonate derivatives and their copper complexes against hormone-dependent (MCF-7), hormone-independent (MDA-MB-231and BT-20) breast cancer and androgen-independent (PC3) prostate cancer cell lines

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ABSTRACT

Retinoids, the derivatives of vitamin A, are known to exhibit anticancer properties through binding to nuclear retinoic acid receptors (RARs). However, several factors such as high lipophilicity, short half life, associated toxicity and appearance of resistant cells during differentiation therapy has limited their therapeutic potential. Here we report synthesis of aryl/heterocyclic analogs of retinoid *trans*-2-octenal with and without copper complexation. The biological activity of these novel synthetic compounds was tested against multiple human cancer cell lines: breast cancer cell lines MCF-7, BT-20, MDA-MB-231 and prostate cancer cell line PC-3, all of which differ in their expression of RAR subunits. The retinoid-derivatives were found to be effective in inhibiting the growth of all the cell lines, particularly those that express RAR α. Synthesis of hydrazonate analogs of retinoic acid and their copper conjugation is therefore an effective strategy to design novel anticancer compounds.

Retinoids are natural or synthetic derivatives of vitamin A, which are small lipophilic molecules capable of modulating cell differentiation, proliferation and exerting antitumoral activities through interaction with specific nuclear receptors and activating or down regulating certain gene expressions [1,2]. Retinoids have been known to possess additional therapeutic benefits such as inhibition of inflammation, keratinization, and cell growth [3]. They bind to nuclear retinoic acid receptors, (RARs) and retinoid X receptors (RXRs), which endows them, with the observed biological activities [4,5]. The changes in the expression of these receptors have been shown to be the possible cause of malignant transformation in human cells [6,7]. Simeone and co-workers have reviewed the ability of the retinoids, viz; all-*trans* retinoic acid (ATRA), 9-*cis*- retinoic acid and N- (4-hydroxyphenyl) retinamide (fenretinide), in inhibiting cell proliferation and promoting apoptosis in breast cancer cells [8]. The compounds have also been found to inhibit growth of prostate and ovarian cancers in animal models [9,10].

Some encouraging reports from the pre-clinical trials have demonstrated the efficacy of using the combination of retinoids and known cytotoxic agents [11–13]. For example, Uslu and co-workers demonstrated that the combination of ATRA and zoledronic acid, is a strong inducer of apoptotic related cell death in ovarian cancer cells. The combination therapy significantly induced pro-apoptotic genes such as tumor necrosis factor receptor super family (TNFRSF), and caspase 4. Several reports have discussed the *in vitro* and pre-clinical models of breast cancer employing MCF-7 cell xenografts, in which ATRA alone or in combination with Tamoxifen induce apoptosis and arrest cancer growth, through regulation of multiple signal transduction pathways [14–16] Recently Ratnam and co-workers have evaluated the efficacy of adjuvant therapy involving estrogen, tamoxifen and ATRA in estrogen sensitive MCF-7, T47D and ZR-75-1 breast cancer cells [17]. Almost every major retinoid is currently in clinical trial by itself or in combination with interferons and estrogen antagonists to treat or prevent the progression of breast cancer [18,19].

Marder and co-workers [20] have recently indicated that structural modifications in the three sub-units of ATRA can lead to subsequent alteration in its binding ability to the RAR and RXR, which in turn can modulate the antiproliferative potential of synthetic retinoids. A recent report by Bisceglie et al., describes preparation and characterization of 9-cis-Retinal thiosemicarbazone and its transition metal complexes which were active against Human leukemic monocyte lymphoma (U937) cells [21]. One of the most biologically active analog of all-trans-retinoic acid (ATRA) is 4-[(1E)-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propen-1-yl] benzoic acid (TTNPB), which exerts antiproliferative effects against breast cancer [22–25].

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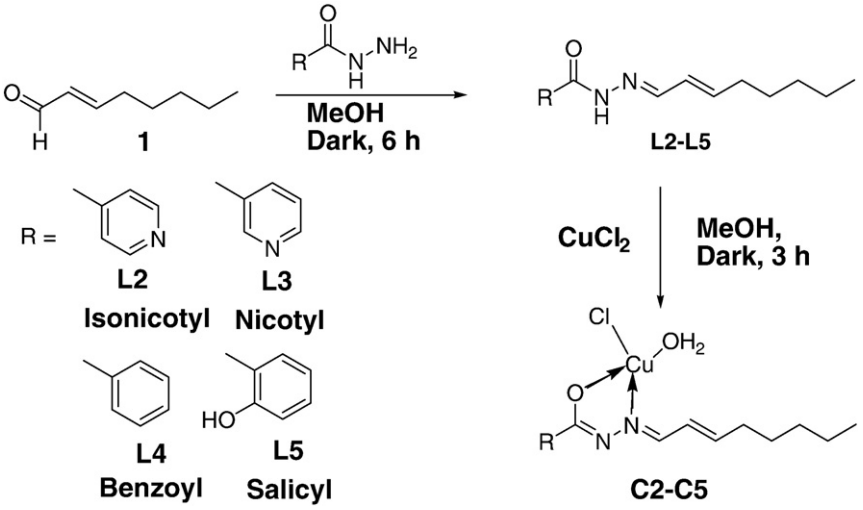
The major limitation in using ATRA compounds for therapeutic purposes include high lipophilicity, short biological half-life, adverse toxicities and appearance of resistant cells during differentiation therapy. Some of these can be alleviated by introducing aryl/heterocyclic functionality into the structural motif of *trans*-2-octenal **(1)** through condensation with hydrazides producing highly polar Schiff base ligands (Scheme 1). Complexation with redox active transition metal ions like copper can influence the intracellular redox milieu of the cancer cells resulting in selective cytotoxicities towards hormone-independent cancer cells [26]. In the present communication we have examined the antiproliferative potential of four ATRA-hydrazonate derivatives and their copper complexes against hormone-dependent (MCF-7) as well as hormone-independent (MDA-MB-231and BT-20) breast cancer cell lines as well as androgen-independent prostate cancer cell line (PC-3) respectively. Our results indicate that these retinoids derivatives are effective in inhibiting the metastasizing cancer cell lines such as BT-20 and PC-3 and show some specificity towards those cancer cell lines in which RARα receptor is involved.

The hydrazonate Schiff base ligands employed in the present work were synthesized in good yields (80–90%) by the condensation reaction of corresponding retinoidal aldehyde with selected hydrazide in methanolic solvent using 1:1 stoichiometry. They yield green colored copper complexes when interacted with $CuCl_2.2H_2O$. Their compositional analyses indicate a general molecular formula as [M(ligand)(H_2O)Cl] and absence of conductivity in DMSO solvent suggesting a non-electrolyte nature for them. The parent *trans*-2-octenal compound (**1**) exhibits the carbonyl stretching frequency at 1691 cm^{-1} which is replaced after the condensation reaction by the imino stretching frequency at 1637–1657 cm^{-1} and additional stretches at 945–957 cm^{-1} due to hydrazinic N-N and C=O linkages respectively. The appearance of the ν(N–H) and ν (C=O) stretching vibrations indicate that in the solid state these ligands essentially exist in the keto form [27]. The broad band at 1603–1633 cm^{-1} arises out of the vibrations of the delocalised (>C=N–N=C<) linkage [28]. The complexes also display a new band in the far-infrared region at 355–330 cm^{-1} which can be assigned to ν(Cu-Cl) stretching frequencies [29(c)]. Following metal complexation the imino stretch is shifted to lower wave number whereas the hydrazinic N-N band shifts to higher wave numbers indicating participation of the azomethine group in copper complexation [29]. Free ligands (L2–L5) exhibit a strong absorption at 284–287 nm attributed to intraligand n-π* transition which undergoes a red shift to 296–302 nm upon metal complexation due to enolization of the ligand. The additional broad absorption observed in the visible region 761–935 nm for copper complexes corresponds to 2B1g→2B2g transition in the square planar environment in these compounds [30,31]. The ^{1}H NMR spectra of all compounds show the proton signal on the azomethine double bond appearing at around 7.53 ppm while a singlet is observed in range of 9.52–9.75 ppm due the NH proton next to the carboxyl of the hydrazone ring. Compound L5 shows the hydroxyl peak at 11.84 ppm due to intramolecular H-bonding with adjacent hydrazinic carbonyl group.

The magnetic susceptibility and EPR data for the complexes C2–C5 are summarized in Table 1. All copper complexes are paramagnetic having magnetic moments between 1.63 and 1.69 BM indicating their mononuclear nature. The calculated EPR parameters yielding the g values $g_{//} > g_{\perp} > g_e$ (2.0023) are consistent with dx^2–y^2 ground state in the square-planar geometry for these copper complexes [32].

The Cu^{+2}/Cu^{+1} reversible redox couple is observed for the present complexes in the range +0.20 to +0.23 V as shown in Table 1. Additional peak in the cyclic voltamogram of the parent ligand at – 0.75 V is due to reduction of the azomethine linkage [33], which is retained upon metal complexation. The difference potentials ($\Delta Ep = Ep_c - Ep_a$) adhere to the Nernstian requirement of 0.059 V while the anodic to cathodic peak current ratio (ip_a/ip_c) values are equal to unity for all copper complexes indicating chemical reversibility of the copper redox couples. Such reversibility implies stereochemical changes from square planar copper(II) to tetrahedral copper(I) geometries [34]. These facile interconversions observed for the present complexes might be of relevance for modulating oxidative stress status of the cancer cells [35].

When hormone-dependent and independent breast and prostate cancer cells were treated with DMSO solutions of present retinol analogs and their copper complexes at different concentrations in MTT assay the heterocyclic hydrazonates were inactive in both cell lines. In case of aryl hydrazonates the unsubstituted benzoyl derivative L4 and C4 show significant inhibition against hormone-dependent breast cancer cell line MCF-7 (RARα,β). None of the derivatives were significantly active against hormone-independent MB-231 (RARγ) as summarized in Fig. 2, while they show promising activity when screened against metastasizing hormone-independent breast cancer cells BT-20 (RAR α) and androgen-independent prostate cancer PC-3 cell line(RAR α,γ) respectively (Fig. 1). Ligand L4 and its copper conjugate C4 showed significant activity lending support to our earlier observation that copper is a key metal which helps in enhancing the biological activity [36,37]. Since the present retinoid derivatives proved to be more efficient in case of metastasizing cancer cells which contain RARα receptor and the hormone-responsive MCF-7 breast cancer cells in which RARα,β is involved, it leads us to believe that they are more specific towards RARα receptors.



Scheme 1. General synthetic scheme for preparation of *trans*-2-octenal hydrazones (L2–L5) and their copper complexes (C2–C5).

Table 1
X-band ESR[a] and electrochemical[b] data for copper complexes (C2–C5).

Compound	X-band ESR parameters					Electrochemical data				
	$g_{//}$	g	$A_{//}$ (G)		f (cm)	E_{pc}(V)	E_{pa}(V)	$E_{1/2}$	ΔE_p	i_{pa}/i_{pc}
			mT	x 10^{-4} cm^{-1}						
C2	2.29	2.05	15.9	169	134	+0.15, −0.69	+0.25	+0.20	0.10	0.93
C3	2.28	2.09	17.0	180	126	+0.18, −0.75	+0.25	+0.22	0.07	1.00
C4	2.27	2.06	13.6	144	157	+0.25, −0.91	+0.38	+0.32	0.13	1.00
C5	2.24	2.05	13.3	139	161	+0.17, −0.93	+0.23	+0.20	0.06	0.96

[a] At 77 K.
[b] At 298 K in DMSO.

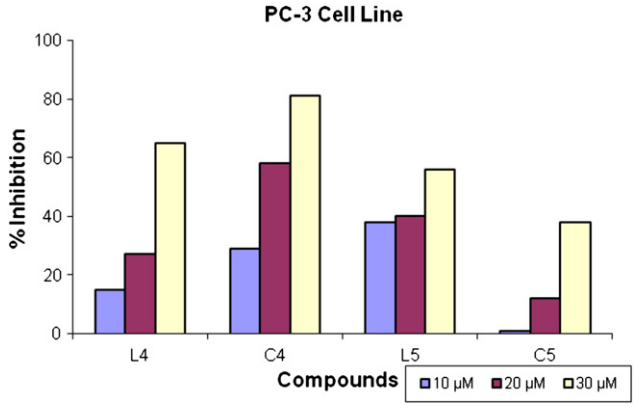


Fig. 1. Cell growth inhibition by *trans*-2-octenal benzoyl hydrazonate L4, *trans*-2-octenal salicylic hydrazone L5 and their copper conjugates (C4–C5) against human prostate cancer (PC-3) cell line.

The present work has shown that copper conjugation of *trans*-2-octenal hydrazones is a promising strategy for evolving effective anticancer agents against hormone -dependent as well as hormone-independent breast and prostate cancers. Although no clear cut trends in the preferences of sub-set receptor selectivity can be seen with present compounds, compound C4 seems to be RARα, β subtype-selective agent and thus shows high sensitivity to both human hormone-dependent and hormone-independent breast cancer cell lines as well as androgen-independent human prostate cancer cell line.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2012.05.027.

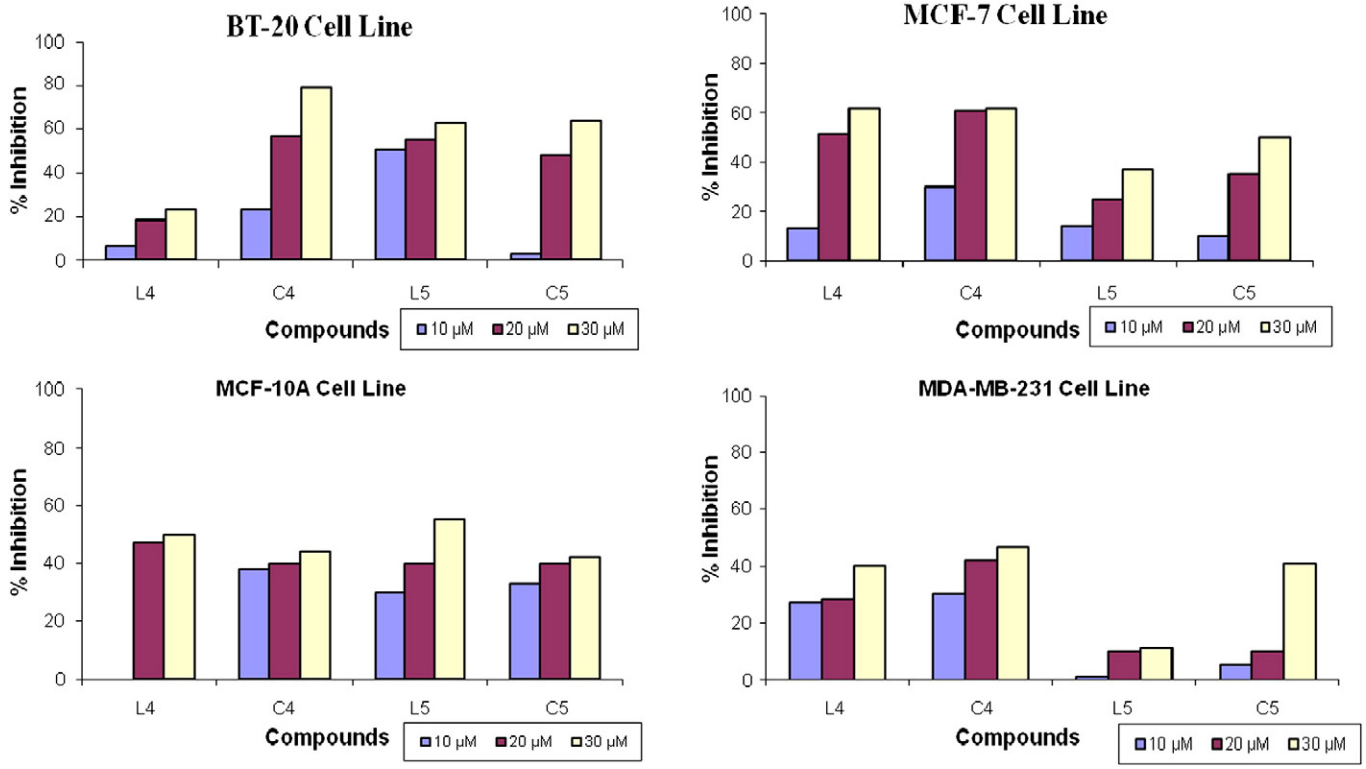


Fig. 2. Antiproliferative effects of *trans*-2-octenal hydrazones (L4–L5) and their copper conjugates (C4–C5) against BT-20 (RARα), MCF-7 (RARα,β), MDA-MB-231 (RARγ) cancer and MCF10A (RARα,β,γ) normal breast cells lines and their specificities of inhibition against retinoid receptor subtypes.

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