RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

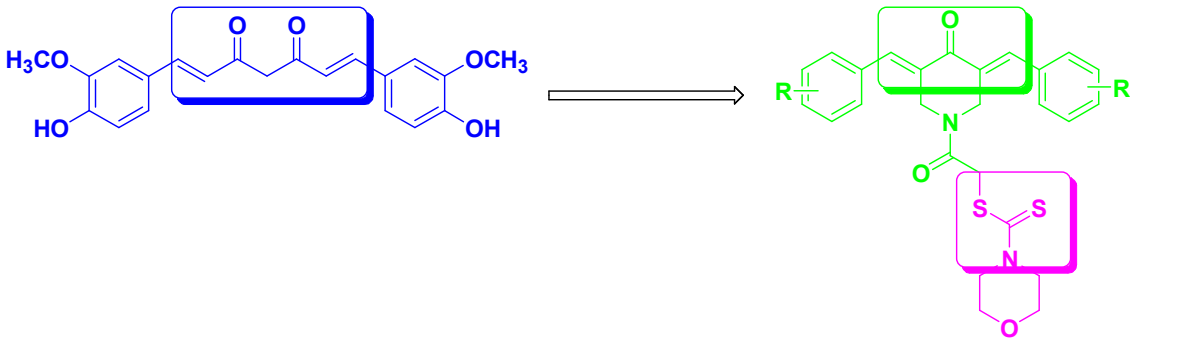
View Article Online
DOI: 10.1039/C4RA03655G

Graphical abstract:

C5-curcuminoid-dithiocarbamate based molecular hybrids: Synthesis, anti-inflammatory and anti-cancer activity evaluation

Amit Anthwal[a,b], Kundan Singh[a], M.S.M. Rawat[b,*] Amit K. Tyagi[c], Bharat B. Aggarwal[c], Diwan S. Rawat[a,*]

The C-5 curcumin-dithiocarbamate analogues were synthesized in search of new molecules with anti-proliferation potential against cancer cells. These new compounds demonstrated higher Anti-proliferation and anti-inflammatory activity against cancer cell lines in comparison to curcumin.



21 Examples: 18 Compounds better than Curcumin

View Article Online
DOI: 10.1039/C4RA03655G

Journal Name

RSCPublishing

COMMUNICATION

C5-curcuminoid-dithiocarbamate based molecular hybrids: Synthesis, anti-inflammatory and anti-cancer activity evaluation

Cite this: DOI: 10.1039/xoxxooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxooooox

www.rsc.org/

Amit Anthwal[a,b], Kundan Singh[a], M.S.M. Rawat[b,*] Amit K. Tyagi[c], Bharat B. Aggarwal[c], Diwan S. Rawat[a,*]

A series of C-5 curcumin bearing morpholine based dithiocarbamates was synthesized. Molinspiration and Osiris software were used for theoretical prediction of physico-chemical properties of these molecules and majority of the hybrids shown theoretical physico-chemical properties similar or better than curcumin. These hybrids (4a-4v) were evaluated for *invitro* cytotoxicity on chronic myeloid leukemia (KBM5) and colon cancer (HCT116) cell lines, and down modulation of TNF-α-induced NF-κB activation at 5μM. Most of the hybrid exhibits higher cytotoxicity against KBM5 cells compared to HCT116 cell lines. Further, all the hybridsshowed potential to suppress the TNF-α-induced NF-κB activation at 5μM KBM5 cells and seventeen hybrids have shown higher potential to inhibit NF-κB activation in comparison to Curcumin.

Introduction

Curcumin, a yellow pigment isolated from dried rhizome of herb *Curcuma longa L.* has been extensively used for centuries in India and China as dietary pigment, spice and traditional medicine.[1]Curcumin has shown to exhibit a wide spectrum of biological and pharmacological activities which includes antioxidant, anti-inflammatory, antibacterial, antiviral.[2] Its therapeutic potential in case of cancer and Alzheimer's disease has also been reported.[2] Additionally, it is evident from various studies carried out on humans[3] and animal models,[4]curcumin is safe even at high doses. In spite of the favourable biological properties and safety profile, poor bioavailability is the main drawback due to which curcumin could not be approved as therapeutic agent.[5] One of the reasons for the poor bioavailability of curcumin is its rapid metabolism into inactive metabolites and rapid elimination from body.[5] It is well documented that β-diketone functionality of curcumin is a substrate for liver aldoketo reductases and this may be one of the reasons for the rapid metabolism of curcumin*in vivo*.[6] In addition, curcumin binds to multiple targets including albumin[7]and thus rarely found unbound/free *in vivo*. These factors were taken into account by various research groups and designed five carbon enone (C-5 curcuminoids) analogues of curcumin replacing sevencarbon enonemoiet(C-7 curcuminoids) in anticipation to retain its activity and but improves bioavailability.[8]Guang et al. carried pharmacokinetic studies on synthetic C-5 curcuminoids, and some of the compounds demonstrated better cytotoxicity and bioavailability both in *vitro* and in *vivo* studies.[9]Dithiocarbamate (DTC) derivatives are another

Dithiocarbamate (DTC) derivatives are another class of compounds known for their anti-tubulin and anticancer activities.[10]Dithiocarbamate (DTCs) based compounds are also known for their antioxidant properties and suppresses the activation of transcription factors NF-κB.[11]The NF-κB plays important role in the development of anti-cancer agents as it regulates the expression of various growth factors and cytokines that are responsible for angiogenesis, tumour growth, metastasis, and anti-apoptosis. Further, activation of NF-κB is a key factor responsible for resistance of tumor cells to chemotherapeutic agents as well as radiation therapy. Suppression of NF-κB is not only an alternative pathway to anticancer therapy, but also linked to suppression of inflammatory responses of cells.[12]Hence, compounds targeting NF-κB are of significant importance in cancer drug development.It has been reported that DTC metal complex has potential to inhibits NF-κB and proteasome, this increases their importance in cancer drug development.[13]A series of dithiocarbamate derivatives has been reported to show anti-tubulin and anticancer activities.[14]It is well known fact that tumour cellshave acidic environment. The acidic conditions triggers decomposition dithiocarbamates to amine and CS_2.[15] The CS_2 formed in the tumour cells due to this decomposition process causes protein cross-linking and is proposed to be most disruptive factor for dithiocarbamate induced toxicity.[16]

In order to develop effective anticancer drug different approaches are being explored and concept of molecular hybrids[17] has drawn attention of the medicinal chemist across the globe. In this approach

COMMUNICATION

View Article Online
DOI: 10.1039/C4RA03559G
Journal Name

two or more pharmacophores are covalently linked together to generate a single molecule. Such molecules may have certain advantages such as: (a) reduced risk of drug resistance, (b) improved potency, (c) improved pharmacokinetic properties, (d) reduced side effect. Encouraged by the concept of molecular hybrids and our own work in this direction,[18] we designed hybrid molecules having C-5 enones covalently linked to dithiocarbamate, an another pharmacophore (fig 1). To this end, herein we report synthesis, theoretical prediction of physicochemical properties, cytotoxicity and inhibition of TNF-α-induced NF-κB activation of newly synthesized hybrids (**4a-4v**). It was found that most of the hybrids exhibited higher cytotoxicity against KBM5 and HCT116 cancer cell linesat 5μM in comparison to curcumin which was used as standard.[19]These compounds were also found to have good potential to inhibit TNF-α-induced NF-κB activation.

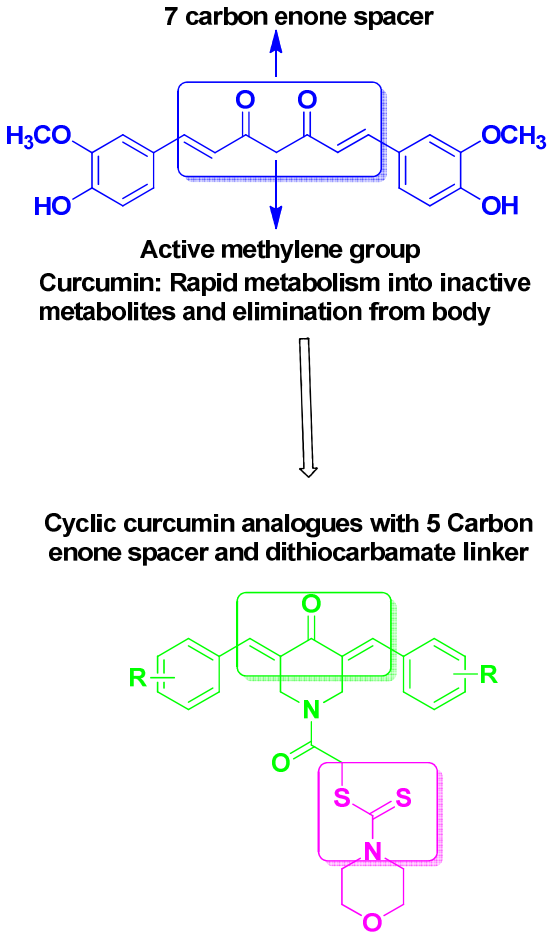
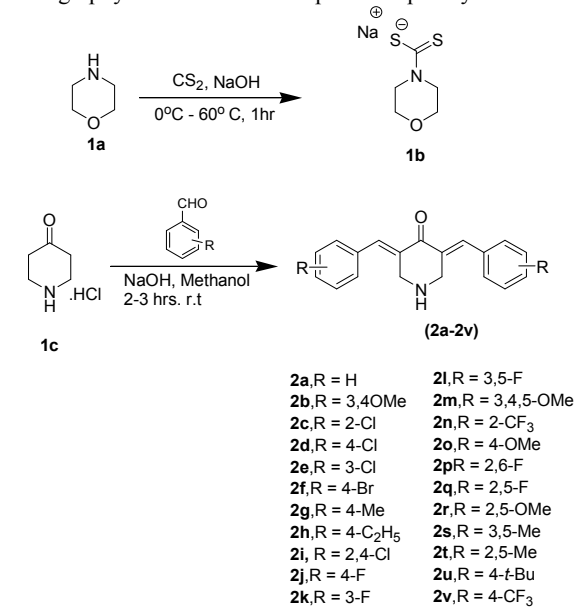


Fig. 1: Modification of β-diketone moiety of curcumin to new cyclic analogues with enhanced activity.

Results and discussion

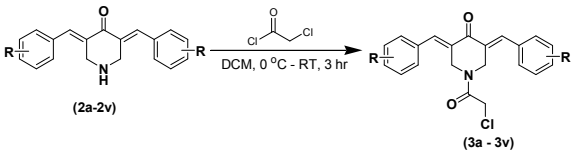
Chemistry

The desired hybrids were synthesized by multistep synthetic protocol. In first step dithiocarbamate salt of morpholine (**1b**) was prepared by the reaction of 45 mmol of morpholine with 45 mmol of CS_2 followed by the addition of 1equiv. of NaOH to obtain desired salt (scheme 1). The desired curcumin analogues (**2a-2v**) were synthesized by aldol condensation reaction of 4-piperidone hydrochloride salt (0.98 mmol) with respective aldehydes (1.96 mmol) in the presence of NaOH using methanol as solvent (scheme 1). The crude compounds (**2a-2v**) were purified by recrystallization from methanol and reacted further with chloro-acetylchloride (0.78 mmol) in the presence of K_2CO_3 (1.95 mmol) using dichloromethane as a solvent, to obtain chloroacetylatedcurcumin analogues (**3a-3v**) in good yields (scheme 2) and were used as such in the subsequent steps. These chloroacetylatedcurcumin analogues (**3a-3v**) (0.45 mmol) were further reacted with **1b** (0.54 mmol) to obtain curcumin-dithiocarbamate based molecular hybrids (**4a-4v**) in good yield (scheme 3). All the compounds were purified by column chromatography and characterized spectroscopically.



2a,R = H
2b,R = 3,4OMe
2c,R = 2-Cl
2d,R = 4-Cl
2e,R = 3-Cl
2f,R = 4-Br
2g,R = 4-Me
2h,R = 4-C_2H_5
2i, R = 2,4-Cl
2j,R = 4-F
2k,R = 3-F
2l,R = 3,5-F
2m,R = 3,4,5-OMe
2n,R = 2-CF_3
2o,R = 4-OMe
2pR = 2,6-F
2q,R = 2,5-F
2r,R = 2,5-OMe
2s,R = 3,5-Me
2t,R = 2,5-Me
2u,R = 4-*t*-Bu
2v,R = 4-CF_3

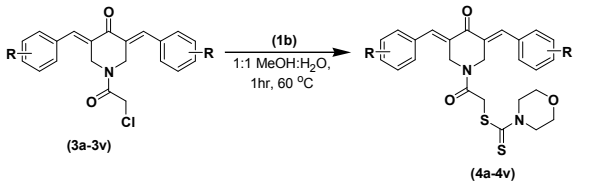
Scheme 1



3a, R = H
3b,R = 3,4OMe
3c,R = 2-Cl
3d,R = 4-Cl
3e,R = 3-Cl
3f,R = 4-Br
3g,R = 4-Me
3h,R = 4-C_2H_5
3i,R = 2,4-Cl
3j,R = 4-F
3k,R = 3-F
3l,R = 3,5-F
3m,R = 3,4,5-OMe
3n,R = 2-CF_3
3o,R = 4-OMe
3p,R = 2,6-F
3q,R = 2,5-F
3r,R = 2,5-OMe
3s,R = 3,5-Me
3t,R = 2,5-Me
3u,R = 4-*t*-Bu
3v,R = 4-CF_3

Scheme 2

View Article Online
DOI: 10.1039/C4RA03855C
COMMUNICATION



4a, R = H
4b,R = 3,4OMe
4c,R = 2-Cl
4d,R = 4-Cl
4e,R = 3-Cl
4f,R = 4-Br
4g,R = 4-Me
4h,R = 4-C_2H_5
4i,R = 2,4-Cl
4j,R = 4-F
4k,R = 3-F
4l,R = 3,5-F
4m,R = 3,4,5-OMe
4n,R = 2-CF_3
4o,R = 4-OMe
4p,R = 2,6-F
4q,R = 2,5-F
4r,R = 2,5-OMe
4s,R = 3,5-Me
4t,R = 2,5-Me
4u,R = 4-*t*-Bu
4v,R = 4-CF_3

Scheme 3

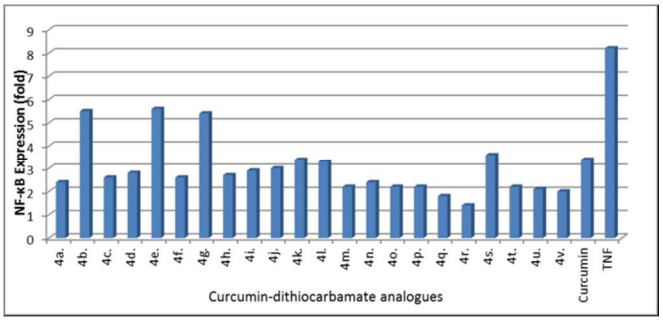


Fig. 2: Down-regulation of TNF-α-induced NF-κB activation in KBM5 cell.

Biology

All the hybrids were subjected to test the cytotoxicity on chronic myeloid leukemia (KBM5) cell line and HCT116 colon cancer cell line. The compounds demonstrated varying degree of cytotoxicity against cancer cell lines but the trend of decrease or increase in cytotoxicity was similar for both the cell lines (Table 1). It was found that the hybrids without any substitution on the aromatic ring (**4a**) were least active.Further, substitution at *meta* or *para*-position of the aromatic ring by electronegative groups like chloro or bromo reduces the activity (**4d, 4e, 4f**) whereas chloro group at *ortho*-position of the aromatic ring enhances cytotoxicity of compounds (**4c, 4i**). The structure activity relationship studies conducted on Curcumin analogues revealed that the substitution of fluoro group at any position of the aromatic ring enhances anticancer activity of the resulting compounds.[20]But in the present study, we observed that hybrids with the fluoro group at *meta* and *para* position (**4j, 4k, 4l**) were more active. The alkyl substitution also enhances the activity of the hybrid molecules (**4s, 4t, 4h**), but the molecules with an alkyl group at *para*-position were the most active (**4g, 4u**). Further introduction ofOMe group on the aromatic ring improves activity of these hybrids (**4r**) but hybrids with OMe group at *meta, para*-positions (**4b, 4m, 4o**) were the most active. It is obvious from results that CF_3 substituent at *para*-position (**4v**) increases the activity to a higher extent compared to that at *ortho*-substutient (**4n**). All compounds were also evaluated for down-regulation of TNF-α-induced NF-κB activation in KBM5 cell. KBM5 cells were pre incubated with 5μM curcumin and C-5 curcumin-dithiocarbamate analogues for 8 hours, and were treated with TNF-α (0.1 nM) for 30 min at 37 °C. The nuclear extracts were prepared and TNF-α-induced NF- κB expression was observed by Electrophoretic Mobility Shift Assay (EMSA). All compounds exhibited a suppression of TNF-α-induced NF- κB activation to different extent (fig 2). The activity of curcumin analogues could not be concluded from inhibition of NF- κB activation as curcumin and its analogues act by multiple pathways, but inhibition of TNF-α-induced NF- κB activation may be one of factors contributing towards the anticancer activity of these compounds. The compound **4e** inhibits NF- κB activation to a lesser extent, so it may be one of the contributing factorfor its poor activity. Further compounds **4n, 4m, 4o, 4p, 4q, 4r, 4v** and **4u** with high cytotoxicity have also demonstrated good potential to inhibit TNF-α-induced NF- κB activation, and this may be one of the factorsfor enhanced activity of these C-5 curcumin-diathiocarbamate analogues.

Table1: Cytotoxicity of the C5-curcuminoid-dithiocarbamate based molecular hybrids(**4a-4v**) on chronic myeloid leukemia (KBM5) and colon cancer (HCT116) cell lines at 5μM.

Compound	Cytotoxicity (% growth inhibition) KBM5	Cytotoxicity (% growth inhibition) HCT116	Compound	Cytotoxicity (% growth inhibition) KBM5	Cytotoxicity (% growth inhibition) HCT116
4a.	61.26 ± 1.82	42.45 ± 1.65	4m.	84.53 ± 0.21	58.58 ± 0.84
4b.	85.46 ± 0.62	59.22 ±0.43	4n.	83.22 ± 1.00	57.67 ± 2.77
4c.	85.50 ± 0.87	59.25 ± 0.60	4o.	85.27 ± 0.63	59.10 ± 1.13
4d.	10.55 ± 1.10	7.31 ± 1.00	4p.	83.15 ± 1.23	57.62 ± 0.85
4e.	53.91 ± 0.47	37.36 ± 1.18	4q.	81.92 ± 1.27	56.77 ± 1.57
4f.	53.47 ± 0.36	37.05 ± 1.71	4r.	79.19 ± 1.43	54.88 ± 1.23
4g.	84.56 ± 1.90	58.60 ± 0.32	4s.	76.99 ± 1.41	53.36 ± 1.21
4h.	76.10 ± 1.27	52.74 ± 0.96	4t.	66.74 ± 2.79	46.25 ± 1.87
4i.	73.53 ± 0.34	50.96 ± 0.24	4u.	85.31 ± 1.53	59.12 ± 1.06
4j.	86.20 ± 1.01	59.74 ± 0.70	4v.	86.88 ± 0.56	60.21 ± 0.39
4k.	87.43 ± 0.73	60.59 ± 0.50	Control	0.00 ± 1.36	0.00 ± 2.33
4l.	87.36 ± 0.87	60.54 ± 0.61	Curcumin	46.00 ± 1.49	46.87 ± 1.03

Theoretical predictions of physico-chemical properties

Molinspiration calculations

Theoreticalphysico-chemical properties and biological activities were calculated using Molinspiration[21,22] software for compounds **4a-4v** (Table 2&3). The logP was calculated as per methodology developed by Molinspiration in which sum of fragment-based contributions and correction factors are considered. According to Lipinski's rules if drug molecules have log P values greater than 5 then there may be a problem for penetration of drug molecule into biomembrane, it is clear from table 2 that many hybridshave log P values less than five. Total polar surface area (TPSA) is calculated on the basis of the methodology published by Ertl et al. as a sum of various fragment contributions.[23] O- and N-centered polar fragments are considered for TPSA calculations. Polar surface area (PSA) has been shown to be an excellent descriptor of various properties like drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood brain barrier penetration. Total polar surface area (TPSA) can be correlated to the hydrogen bonding potential of a compound as it is described as an area occupied by electronegative elements like oxygen and nitrogen, but it is also reported that if TPSA exceeds 140 a then the molecule may exhibit poor intestinal absorption. [23] It is obvious from table 2 that all the hybrids (**4a-4v**) have TPSA less than 140 A°. The molecule may

COMMUNICATION

View Article Online
DOI: 10.1039/C4RA03559G
Journal Name

show poor bioavailability if it violates two or more Rules of 5.[24]Druglikeness may be considered as a complex balance of various structural features and molecular properties which predicts similarity of a particular molecule with the known a drug. Calculation of druglikeness score in terms of GPCR ligand activity, ion channel modulators, kinase inhibitors, nuclear receptor ligand activity, protease inhibitors and other enzyme inhibitors is based on Molinspiration technology. It is found that most of marketed drugs have negative values or very low positive values of score for above mentioned enzyme targets. It is very clear from table 3 that all compounds (**4a-4v**) have negative values of enzyme target scores.

Table 2: Molinspirationcalculations of the hybrids (**4a-4v**).

Comp.	Mol. Wt.	m_iLogP	TPSA	OH-NH Interact.	N viol.	Vol.
4a.	478	4.08	49	0	0	424
4b.	598	3.373	86	0	1	526
4c.	546	4.981	49	0	1	451
4d.	546	5.436	49	0	2	451
4e.	546	5.388	49	0	2	451
4f.	634	5.698	49	0	2	460
4g.	506	4.977	49	0	1	457
4h.	534	5.909	49	0	2	491
4i.	614	6.289	49	0	2	478
4j.	514	4.407	49	0	1	434
4k.	514	4.359	49	0	1	434
4l.	550	4.591	49	0	1	444
4m.	658	3.342	105	0	2	577
4n.	614	5.415	49	0	2	487
4o.	538	4.193	68	0	1	475
4p.	550	4.183	49	0	1	444
4q.	550	4.231	49	0	1	444
4r.	598	3.803	86	0	1	526
4s.	534	5.730	49	0	2	490
4t.	534	5.371	49	0	2	490
4u.	590	7.492	49	0	2	557
4v.	614	5.871	49	0	2	487
Curcumin	368	2.303	93	2	0	332

Comp.	GPCR	ICM	KI	NRL	PI	EI
4a.	-0.31	-0.81	-0.84	-0.75	-0.34	-0.28
4b.	-0.31	-1.01	-0.81	-0.74	-0.34	-0.38
4c.	-0.31	-0.79	-0.89	-0.71	-0.37	-0.32
4d.	-0.30	-0.78	-0.81	-0.72	-0.35	-0.29
4e.	-0.29	-0.77	-0.82	-0.73	-0.35	-0.29
4f.	-0.37	-0.83	-0.83	-0.78	-0.40	-0.32
4g.	-0.33	-0.83	-0.83	-0.73	-0.36	-0.30
4h.	-0.27	-0.78	-0.79	-0.65	-0.30	-0.25
4i.	-0.29	-0.80	-0.85	-0.68	-0.36	-0.31
4j.	-0.29	-0.78	-0.77	-0.69	-0.34	-0.28
4k.	-0.28	-0.78	-0.71	-0.68	-0.32	-0.27
4l.	-0.27	-0.78	-0.71	-0.64	-0.28	-0.25
4m.	-0.47	-1.35	-1.03	-1.04	-0.39	-0.63
4n.	-0.23	-0.89	-0.74	-0.62	-0.27	-0.33
4o.	-0.31	-0.84	-0.78	-0.68	-0.35	-0.29
4p.	-0.28	-0.83	-0.77	-0.66	-0.34	-0.30
4q.	-0.28	-0.78	-0.79	-0.66	-0.32	-0.27
4r.	-0.31	-1.02	-0.83	-0.72	-0.33	-0.39
4s.	-0.30	-0.83	-0.78	-0.68	-0.34	-0.29
4t.	-0.29	-0.86	-0.79	-0.69	-0.35	-0.30
4u.	-0.25	-0.91	-0.78	-0.65	-0.28	-0.33
4v.	-0.24	-0.90	-0.74	-0.62	-0.26	-0.34
Curcumin	-0.06	-0.20	-0.26	0.12	-0.14	-0.08

Table 3: Molinspiration based drug likeness of the hybrids (**4a-4v**).

GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor;NRL: nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor.

Osiris calculations [21,25]

The Osiris [21,25] calculations are tabulated in table 4. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physico-

View Article Online
DOI: 10.1039/C4RA03655G

chemical properties (ClogP, solubility, drug likeness and drug score) of compounds (**4a-4v**) are calculated by the methodology developed by Osiris. The log P value of most of the compounds isless than 5 which is positive indication. Except few, most of the compounds demonstrated low toxicity risks. Also, many compounds have druglikeness and drug score comparable or better than curcumin (table 4).

Table 4: Osiris calculations of the hybrids (**4a-4v**).

Comp.	MUT	TUMO	IRRI	REP	Mol. Wt.	ClogP	LogS	DL	D-S
4a.	-	-	-	-	478	4.44	-4.05	2.97	0.54
4b.	-	-	-	-	598	4.02	-4.12	5.89	0.45
4c.	-	-	-	-	546	5.66	-5.52	4.57	0.31
4d.	+	-	-	-	546	5.66	-5.52	4.72	0.25
4e.	-	-	-	-	546	5.66	-5.52	3.98	0.31
4f.	-	-	-	-	634	5.83	-5.72	2.12	0.24
4g.	+	-	-	-	506	5.07	-4.74	2.64	0.33
4h.	-	-	-	-	534	5.78	-5.05	3.83	0.34
4i.	-	-	-	-	614	6.89	-6.99	4.95	0.19
4j.	-	-	-	+	514	4.55	-4.68	3.07	0.36
4k.	-	-	-	+	514	4.55	-4.68	1.36	0.33
4l.	-	-	-	-	550	4.67	-5.30	0.40	0.30
4m.	-	-	-	-	658	3.81	-4.16	6.87	0.42
4n.	-	-	-	-	614	5.96	-5.60	-5.49	0.13
4o.	-	-	-	-	538	4.23	-4.08	4.24	0.50
4p.	-	-	-	-	550	4.67	-5.30	1.33	0.34
4q.	-	-	-	-	550	4.67	-5.30	1.16	0.34
4r.	-	-	-	-	598	4.02	-4.12	4.58	0.45
4s.	+	-	-	-	534	5.70	-5.42	0.01	0.19
4t.	-	+	-	-	534	5.70	-5.42	1.88	0.24
4u.	-	-	-	-	590	7.47	-6.37	0.91	0.17
4v.	-	-	-	-	614	5.96	-5.60	-3.30	0.13
Curcumin	-	-	-	-	368	2.97	-3.62	-3.95	0.39

MUT: Mutagenic; TUMO: Tuorogenic; IRRI: Irritant; REP: Reproductive effective. Mol. Wt.: Molecular weight in g/mol; C log P: log of octanol/water partition coefficient; S: Solubility; DL: Drug likness; D-S: Drug-score. - : Low risk; +: medium risk; ++: high risk

Experimental section

General

All the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as such. Thin layer chromatography (Merck TLC silica gel 60 F_{254}) was used to monitor the progress of the reactions. The compounds were purified when needed by silica gel column (60-120 mesh). Melting points were determined on EZ-Melt automated melting point apparatus, Stanford Research systems and are uncorrected. IR (chloroform/film) spectra were recorded using Perkin-Elmer FT-IR spectrophotometer and values are expressed as υ_{max} cm^{-1}. Mass Spectra were recorded in waters micromass LCT Mass Spectrometer.The ^1H NMR and ^{13}C NMR spectra were recorded on Jeol ECX spectrospin at 400 MHz and 100 MHz respectively, in deuterated solvents with TMS as an internal standard. Chemical shift values are recorded on δ ppm and the coupling constants *J* are in Hz.

Typical procedure for synthesis of sodium morpholine-4-carbodithioate (1b):
To a stirred solution of morpholine (45mmol)(**1a**) in methanol at 0 ^oC (ice bath) was added CS_2 (45mmol) slowly drop wise (exothermic reaction). On addition of CS_2 solid gets precipitated and reaction mixture was further stirred for 30 minutes at room temperature. After 30 minutes, NaOH (45mmol) was added to the reaction mixture and stirring was continued at 60^oC for 1 hr. After completion of the reaction, reaction mixture was cooled to room temperature; and solvent was evaporated to 1/3 of initial volume by rotary evaporator. The solid thus obtained was filtered, dried and used for subsequent reaction (70% yield) (scheme 1).

General procedure for synthesis of compounds 2a-2v:
Mixture of respective aromatic aldehyde (1.96 mmol) and 4-piperidone (**1c**) (0.98mmol) were dissolved in methanol in a round bottom flask and stirred for few minutes. To this, 40% NaOH solution was added drop wise to the reaction mixture. The reaction mixture was then allowed to stir at room temperature for 3 hrs. After 3 hrs the precipitate thus obtained was filtered, washed with water, dried and recrystallized from methanol to get pure product (**2a-2v**) (68-81% yield) (scheme 1).

General procedure for synthesis of compounds 3a-3v:

The mixture of **2a-2v** (0.65mmol) and anhydrous K_2CO_3 (1.95mmol) in dichloromethane was maintained at 0^oC (ice bath). To this cooled mixture, chloroacetylchloride (0.78mmol) was added drop wise. After the complete addition of chloroacetylchloride the reaction mixture was stirred further for 3 hrs. After completion of the reaction solvent was evaporated and the residue thus obtained was washed with water, filtered and dried. The products obtained were pure enough to be used for subsequent step(74-80 % yield) (scheme 2).

General procedure for synthesis of compounds 4a-4v:
The mixture of compound (**3a-3v**) (0.45mmol)and sodium morpholine-4-carbodithioate (**1b**) (0.54mmol)in methanol: water 1:1 was heated at 60^oC for 0.5 hrs.[26] The solid thus obtained was filtered, washed with water, dried and recrystallized from methanol to obtain pure product (4a-4v) (scheme 3).

(*3E,5E*)-1-(2-chloroacetyl)-3,5-bis(3,4,5-trimethoxy benzylidene)piperidin-4-one (3m):
Yield: 79%; ^1H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 18H), 3.97 (s, 2H), 4.89 (s, 2H), 4.94 (s, 2H), 6.64 (s, 2H), 6.72 (s, 2H), 7.78 (s, 1H), 7.81 (s, 1H).
2-((*3E,5E*)-3,5-dibenzylidene-4-oxopiperidin-1-yl)-2-oxoethyl morpholine-4-carbodithioate (4a):
Yield: 78% (pale yellow solid); mp 169-170 ^oC; IR ($CHCl_3$, cm^{-1}): 2969, 2845, 2363, 1654, 1611, 1429, 1266, 1236, 986, 762, 694; ^{1}HNMR (400 MHz, $CDCl_3$): δ 3.70 (t, 4H, *J* = 4.4 Hz), 3.88 (brs, 2H), 4.17 (s, 4H), 4.95 (s, 4H), 7.38-7.48 (m, 10H), 7.85 (s, 1H), 7.90 (s, 1H); TOF-MS *m/z* 501.0928 $[M+23]^+$, Anal. Calcd for $C_{26}H_{26}N_2O_3S_2$: C, 65.24; H, 5.48; N, 5.85. Found: C, 64.88; H, 5.95; N, 5.67
2-((*3E,5E*)-3,5-bis(3,4-dimethoxybenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4b):
Yield: 66% (pale yellow solid; mp 175-176^oC; IR ($CHCl_3$, cm^{-1}): 2934, 2838, 2363, 1638, 1596, 1420, 1282, 1255, 946, 815; ^{1}H NMR(400MHz,$CDCl_3$): δ3.70(t, 4H, *J* = 5.1Hz), 3.93-3.94 (m, 14H), 4.23(s, 4H), 4.96(s, 4H), 6.91(d, 1H, *J* = 5.1Hz), 6.96 (d, 2H, *J*

View Article Online
DOI: 10.1039/C4RA03559G Journal Name

= 5.1Hz), 7.01 (s, 1H), 7.04-7.09 (m, 2H), 7.78 (s, 1H), 7.82 (s, 1H); TOF-MS *m/z* 621.1807 $[M+23]^+$, Anal. Calcd for $C_{30}H_{34}N_2O_7S_2$: C, 60.18; H, 5.72; N, 4.68 Found: C, 60.34; H, 5.78; N, 4.71.

2-((*3E,5E*)-3,5-bis(2-chlorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4c):
Yield: 71% (yellow solid); mp 190-191^oC; IR ($CHCl_3$, cm^{-1}): 2954, 2902, 2863, 2362, 1654, 1620, 1460, 1269, 994, 755; ^1H NMR (400MHz, $CDCl_3$): δ 3.71(s, 4H), 3.90 (brs, 2H), 4.19 (s, 4H), 4.77 (d, 4H, *J* = 6.6Hz), 7.31-7.5 (m, 4H), 7.40-7.48 (m, 4H), 8.02 (s, 2H); TOF-MS *m/z* 569.0605 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_3S_2$: C, 57.04; H, 4.42; N, 5.12. Found: C, 57.31; H, 4.48; N, 5.01

2-((*3E,5E*)-3,5-bis(4-chlorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4d):
Yield: 74% (pale yellow solid); mp 178-179^oC; IR ($CHCl_3$, cm^{-1}): 2938, 2364, 1654, 1602, 1507, 1297, 808; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.71 (t, 4H, *J* = 5.1Hz), 3.91 (brs, 2H), 4.18 (s, 4H), 4.90 (s, 4H), 7.36 (d, 2H, *J* = 8.8Hz), 7.40-7.46 (m, 6H), 7.77 (s, 1H), 7.82 (s, 1H); TOF-MS *m/z* 569.0605 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_3S_2$: C, 57.04; H, 4.42; N, 5.12. Found: C, 57.28; H, 4.43; N, 5.07

2-((*3E,5E*)-3,5-bis(3-chlorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4e):
Yield: 72% (yellow solid); mp 181-182^oC; IR ($CHCl_3$, cm^{-1}): 2965, 2923, 2859, 2362, 1638, 1610, 1458, 1269, 997, 790, 684; ^1H NMR (400MHz, $CDCl_3$): δ 3.71 (s, 4H), 3.89 (brs, 2H), 4.17 (s, 4H), 4.91 (s, 4H), 7.31-7.40 (m, 8H), 7.75 (s, 1H), 7.80 (s, 1H); TOF-MS *m/z* 569.0605 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}Cl_2N_2O_3S_2$: C, 57.04; H, 4.42; N, 5.12. Found: C, 57.29; H, 4.45; N, 5.05

2-((*3E,5E*)-3,5-bis(4-bromobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4f):
Yield: 77% (pale yellow solid); mp 237-238^oC; IR ($CHCl_3$, cm^{-1}): 2920, 2963, 2851, 2365, 1654, 1609, 1487, 1267, 824; ^{1}HNMR (400 MHz, $CDCl_3$): δ 3.71 (brs, 4H), 3.88 (s, 2H), 4.20 (s, 4H), 4.89 (s, 4H), 7.29 (d, 2H, *J* = 8.8Hz), 7.33 (d, 2H, *J* = 8.0Hz), 7.56 (d, 2H, *J* = 8.0Hz), 7.61 (d, 2H, *J* = 8.0Hz), 7.75 (s, 1H), 7.80 (s, 1H); TOF-MS *m/z* 656.9595 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}Br_2N_2O_3S_2$: C, 49.07; H, 3.80; N, 4.40. Found: C, 49.09; H, 3.69; N, 4.51.

2-((*3E,5E*)-3,5-bis(4-methylbenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4g):
Yield: 81% (pale yellow solid); mp 158-159^oC; IR ($CHCl_3$, cm^{-1}): 2855, 2364, 1602, 1430, 1265, 992, 815; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 2.41 (s, 3H), 3.69 (t, 4H, *J* = 5.1Hz), 3.89 (brs, 2H), 4.19 (s, 4H), 4.93 (d, 4H, *J* = 4.4Hz), 7.22 (d, 2H, *J* = 7.3Hz), 7.28 (d, 2H, *J* = 7.3Hz), 7.33 (d, 2H, *J* = 8.0Hz), 7.37 (d, 2H, *J* = 8.0Hz), 7.80 (s, 1H), 7.85 (s, 1H); TOF-MS *m/z* 529.0775 $[M+23]^+$, Anal. Calcd for $C_{28}H_{30}N_2O_3S_2$: C, 66.37; H, 5.97; N, 5.53. Found: C, 66.42; H, 5.93; N, 5.60.

2-((*3E,5E*)-3,5-bis(4-ethylbenzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl morpholine-4-carbodithioate (4h):
Yield: 76% (pale yellow solid); mp 161-162^oC; IR ($CHCl_3$, cm^{-1}): 2965, 2929, 2850, 2365, 1654, 1603, 1458, 1268, 1228, 996, 828; ^{1}HNMR (400MHz, $CDCl_3$): δ 1.23-1.29 (m, 6H), 2.65-2.73 (m, 4H), 3.69 (t, 4H, *J* = 5.1Hz), 3.93 (brs, 2H), 4.20 (s, 4H), 4.94 (s, 4H), 7.24 (s, 1H), 7.30 (d, 2H, *J* = 8.0Hz), 7.36 (d, 3H, *J* = 8.0Hz), 7.40 (d, 2H, *J* = 8.0Hz), 7.82 (s, 1H), 7.86 (s, 1H); TOF-MS *m/z* 557.2011 $[M+23]^+$, Anal. Calcd for $C_{30}H_{34}N_2O_3S_2$: C, 67.38; H, 6.41; N, 5.24. Found: C, 67.29; H, 6.34; N, 5.28.

2-((*3E,5E*)-3,5-bis(2,4-dichlorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4i):
Yield: 75% (pale yellow solid); mp 163-164^oC; IR ($CHCl_3$, cm^{-1}): 3062, 2917, 2855, 2364, 1653, 1616, 1467, 1271, 1233, 995, 844; ^1H NMR (400MHz, $CDCl_3$): δ 3.73-3.88 (m, 6H), 4.14 (s, 4H), 4.74 (s, 4H), 7.22 (d, 1H,*J* = 8.8Hz), 7.30 (d, 1H, *J* = 7.3Hz), 7.32 (d, 1H, *J* = 8.8Hz), 7.37 (d, 1H, *J* = 8.8Hz), 7.48 (s, 1H), 7.52 (s, 1H), 7.93 (s, 2H); TOF-MS *m/z* 636.9826 $[M+23]^+$, Anal. Calcd for $C_{26}H_{22}Cl_4N_2O_3S_2$: C, 50.66; H, 3.60; N, 4.54. Found: C, 50.58; H, 3.71; N, 4.60.

2-((*3E,5E*)-3,5-bis(4-fluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4j):
Yield: 69% (pale yellow solid); mp 163-164^oC; IR ($CHCl_3$, cm^{-1}): 2972, 2856, 2365, 1647, 1599, 1414, 1269, 1234, 990,840; ^1H NMR (400MHz, $CDCl_3$): δ 3.73(s, 4H), 3.88 (brs, 2H), 4.19 (s, 4H), 4.91 (s, 4H), 7.10 (d, 1H, *J* = 8.8Hz), 7.14 (d, 1H, *J* = 2.9Hz), 7.18 (d, 2H, *J* = 8.0Hz), 7.43-7.45 (m, 4H), 7.79 (s, 1H), 7.83 (s, 1H); TOF-MS *m/z* 537.1196 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}F_2N_2O_3S_2$: C, 60.68; H, 4.70; N, 5.44. Found: C, 60.62; H, 4.67; N, 5.52.

2-((*3E,5E*)-3,5-bis(3-fluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4k):
Yield: 71% (pale yellow solid); mp 198-199^oC; IR ($CHCl_3$, cm^{-1}): 2853, 2364, 1653, 1617, 1430, 1269, 1227, 996, 779; ^{1}HNMR (400 MHz, $CDCl_3$): δ 3.71(t, 4H, *J* = 4.4Hz), 3.91 (brs, 2H), 4.18 (s, 4H), 4.92 (s, 4H), 7.10-7.16 (m, 4H), 7.20-7.25 (m, 2H), 7.39-7.46 (m, 2H),7.78 (s, 1H), 7.83 (s, 1H); TOF-MS *m/z* 537.1196 $[M+23]^+$, Anal. Calcd for $C_{26}H_{24}F_2N_2O_3S_2$: C, 60.68; H, 4.70; N, 5.44. Found: C, 60.62; H, 4.69; N, 5.51.

2-((*3E,5E*)-3,5-bis(3,5-difluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4l):
Yield: 70% (pale yellow solid); mp 205-206^oC; IR ($CHCl_3$, cm^{-1}): 3065, 2864,2365, 1641, 1618, 1430, 1265, 1238, 994, 858; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.72(t, 4H, *J* = 4.4Hz), 3.91 (brs, 2H), 4.18 (s, 4H), 4.89 (d, 4H, *J* = 8.0Hz), 6.84 (d, 1H, *J* = 8.8Hz), 6.88 (d, 1H, *J* = 8.0Hz), 6.93 (d, 2H, *J* = 5.9Hz), 6.97 (d, 2H, *J* = 5.9Hz), 7.71 (s, 1H), 7.76 (s, 1H); TOF-MS *m/z* 573.1008 $[M+23]^+$, Anal. Calcd for $C_{26}H_{22}F_4N_2O_3S_2$: C, 56.72; H, 4.03; N, 5.09. Found: C, 56.79; H, 4.14; N, 5.02.

2-oxo-2-((*3E,5E*)-4-oxo-3,5-bis(3,4,5-trimethoxybenzylidene) piperidin-1-yl)ethylmorpholine-4-carbodithioate (4m) :
Yield: 72% (pale yellow solid); mp 181-182 ^oC; IR ($CHCl_3$, cm^{-1}): 2938, 2839, 2365, 1642, 1605, 1505, 1419, 1252, 997, 836; ^1H NMR (400 MHz, $CDCl_3$): δ 3.71 (t, 4H, *J* = 5.1Hz), 3.91-3.98 (m, 20H), 4.23 (s, 4H), 4.96 (s, 2H), 4.99 (s, 2H), 6.65 (s, 2H), 6.71 (s, 2H), 7.77 (s, 1H), 7.80 (s, 1H); TOF-MS *m/z* 681.2019 $[M+23]^+$, Anal. Calcd for $C_{32}H_{38}N_2O_9S_2$: C, 58.34; H, 5.81; N, 4.25. Found: C, 58.42; H, 5.76; N, 4.31.

2-oxo-2-((*3E,5E*)-4-oxo-3,5-bis(2-(trifluoromethyl)benzylidene) piperidin-1-yl)ethylmorpholine-4-carbodithioate (4n):
Yield: 79% (pale yellow solid); mp 184-185^oC; IR($CHCl_3$, cm^{-1}): 2857, 2363, 1654, 1618, 1430, 1315, 1163, 991, 771; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.72 (t, 4H, *J* = 5.1Hz), 3.91 (brs, 2H), 4.06 (s, 4H), 4.64 (s, 4H), 7.33 (d, 1H, *J* = 7.3Hz), 7.46 (d, 2H, *J* = 7.3Hz), 7.54-7.65 (m, 3H), 7.73 (d, 1H, *J* = 8.0Hz), 7.79 (d, 1H, *J* = 7.3Hz), 8.05 (s, 1H), 8.09 (s, 1H); ^{13}C-NMR (100MHz, $CDCl_3$): δ39.24, 43.18, 46.67, 65.92, 125.76, 126.77, 127.91, 128.21, 130.09, 131.23, 133.13, 134.62, 165.96, 185.59, 194.63; TOF-MS *m/z* 637.1133 $[M+23]^+$, Anal. Calcd for $C_{28}H_{24}F_6N_2O_3S_2$: C, 54.72; H, 3.94; N, 4.56. Found: C, 54.78; H, 3.87; N, 4.61.

View Article Online
DOI: 10.1039/C4RA03655G
COMMUNICATION

2-((*3E,5E*)-3,5-bis(4-methoxybenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4o):
Yield: 77% (yellow solid); mp 174-175°C; IR ($CHCl_3$, cm^{-1}): 2964, 2838, 2363, 1635, 1598, 1510, 1259, 1167, 998, 830; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.69 (t, 4H, *J* = 4.8Hz), 3.85 (d, 8H, *J* = 8.0Hz), 4.22 (s, 4H), 4.92 (d, 4H, *J* = 4.4Hz), 6.94 (d, 2H, *J* = 8.0Hz), 6.98 (d, 2H, *J* = 8.0Hz), 7.40 (d, 2H, *J* = 8.0Hz), 7.44 (d, 2H, *J* = 8.0Hz), 7.78 (s, 1H), 7.83 (s, 1H); TOF-MS *m/z* 561.1596 $[M+23]^+$, Anal. Calcd for $C_{28}H_{30}N_2O_5S_2$: C, 62.43; H, 5.61; N, 5.20. Found: C, 62.51; H, 5.69; N, 5.24.

2-((*3E,5E*)-3,5-bis(2,6-difluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4p):
Yield: 71% (pale yellow solid); mp 203-204°C; IR ($CHCl_3$, cm^{-1}): 2964, 2903, 2839, 2363, 1648, 1629, 1463, 1264, 1233, 997, 786; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.71 (s, 4H), 3.89 (brs, 2H), 4.12 (s, 4H), 4.61 (s, 4H), 6.95 (d, 2H, *J* = 7.3Hz), 6.98 (d, 1H, *J* = 7.3Hz), 7.02 (d, 1H, *J* = 8.0Hz), 7.34-7.41 (m, 2H), 7.74 (s, 2H); TOF-MS *m/z* 573.1008 $[M+23]^+$, Anal. Calcd for $C_{26}H_{22}F_4N_2O_3S_2$: C, 56.72; H, 4.03; N, 5.09. Found: C, 56.80; H, 4.16; N, 5.04.

2-((*3E,5E*)-3,5-bis(2,5-difluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4q):
Yield: 69% (pale yellow solid); mp 192-193°C; IR ($CHCl_3$, cm^{-1}): 3066, 2966, 2856, 2365, 1654, 1623, 1484, 1267, 1236, 987, 876; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.72 (s, 4H), 3.92 (brs, 2H), 4.17 (s, 4H), 4.79 (s, 4H), 7.03-7.10 (m, 6H), 7.83 (s, 2H); TOF-MS *m/z* 573.1008 $[M+23]^+$, Anal. Calcd for $C_{26}H_{22}F_4N_2O_3S_2$: C, 56.72; H, 4.03; N, 5.09. Found: C, 56.77; H, 4.13; N, 5.03.

2-((*3E,5E*)-3,5-bis(2,5-dimethoxybenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4r):
Yield: 73% (yellow solid); mp 208-209°C; IR ($CHCl_3$, cm^{-1}): 2937, 2833, 2363, 1646, 1606, 1491, 1458, 1270, 1227, 994, 805; ^{1}HNMR (400MHz, $CDCl_3$): δ 3.69-3.88 (m, 18H), 4.15 (s, 4H), 4.76 (s, 2H), 4.80 (s, 2H), 6.75 (d, 1H, *J* = 2.2Hz), 6.83 (d, 1H, *J* = 8.8Hz), 6.87 (d, 2H, *J* = 4.4Hz), 6.90 (s, 1H), 6.93 (dd, 1H, J_1= 2.9, Hz, J_2 = 5.9Hz), 7.98 (s, 1H), 8.01 (s, 1H); TOF-MS *m/z* 621.1807 $[M+23]^+$, Anal. Calcd for $C_{30}H_{34}N_2O_7S_2$: C, 60.18; H, 5.72; N, 4.68 Found: C, 60.32; H, 5.76; N, 4.73.

2-((*3E,5E*)-3,5-bis(3,5-dimethylbenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4s):
Yield: 65% (pale yellow solid); mp 151-152°C; IR ($CHCl_3$, cm^{-1}): 2916, 2852, 2365, 1647, 1610, 1426, 1270, 1233, 994, 848; ^{1}H NMR (400MHz, $CDCl_3$): δ 2.34 (s, 6H), 2.37 (s, 6H), 3.69 (t, 4H, *J* = 4.8Hz), 3.88 (brs, 2H), 4.20 (s, 4H), 4.92 (s, 4H), 7.02 (s, 2H), 7.04 (s, 2H), 7.05 (s, 2H) 7.77 (s, 1H), 7.81 (s, 1H); ^{13}C-NMR (100MHz, DMSO-d_6): δ21.33, 21.30, 39.47, 44.09, 47.03, 65.98, 128.15, 128.32, 130.87, 131.31, 131.46, 134.15, 134.40, 137.90, 138.09, 138.37, 138.81, 166.14, 186.41, 195.46; TOF-MS *m/z* 557.2011$[M+23]^+$, Anal. Calcd for $C_{30}H_{34}N_2O_3S_2$: C, 67.38; H, 6.41; N, 5.24. Found: C, 67.25; H, 6.51; N, 5.32.

2-((*3E,5E*)-3,5-bis(2,5-dimethylbenzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4t):
Yield: 73% (pale yellow solid); mp 151-152°C; IR ($CHCl_3$, cm^{-1}): 2919, 2859, 2364, 1648, 1617, 1423, 1269, 1228, 996, 817; ^{1}HNMR (400MHz, $CDCl_3$): δ 2.30 (s, 3H), 2.32 (s, 6H), 2.37 (s, 3H), 3.71 (t, 4H, *J* = 4.4Hz), 3.90 (brs, 2H), 4.09 (s, 4H), 4.76 (brs, 4H), 6.97 (s, 1H), 7.04 (s, 1H), 7.07 (d, 1H, *J* = 7.3Hz), 7.11 (d, 2H, *J*= 8.8Hz), 7.16 (d, 1H, *J* = 7.3Hz), 7.98 (s, 1H), 8.00 (s, 1H); ^{13}C-NMR (100MHz, DMSO-d_6): δ19.75, 19.88, 21.22, 21.28, 39.35, 43.91, 47.08, 66.12, 130.01, 130.11, 130.65, 130.91, 132.88, 133.64, 133.72, 135.39, 135.48, 136.01, 136.07, 166.08, 186.38, 195.14; TOF-MS *m/z* 557.2011 $[M+23]^+$, Anal. Calcd for $C_{30}H_{34}N_2O_3S_2$: C, 67.38; H, 6.41; N, 5.24. Found: C, 67.27; H, 6.49; N, 5.33.

2-((*3E,5E*)-3,5-bis(4-(tert-butyl)benzylidene)-4-oxopiperidin-1-yl)-2-oxoethylmorpholine-4-carbodithioate (4u):
Yield: 80% (pale yellow solid); mp 171-172°C; IR ($CHCl_3$, cm^{-1}): 2864, 2365, 1647, 1420, 1327, 1270, 1119, 994, 849; ^{1}HNMR (400MHz, $CDCl_3$): δ 1.33(s, 9H), 1.35 (s, 9H), 3.70 (t, 4H, *J* = 5.1Hz), 3.97 (brs, 2H), 4.22 (s, 4H), 4.95 (s, 4H), 7.39 (d, 3H, *J* = 8.8Hz), 7.42-7.45 (m, 3H), 7.49 (d, 2H, *J* = 8.0Hz), 7.83 (s, 1H), 7.86 (s, 1H); TOF-MS *m/z* 613.2637 $[M+23]^+$, Anal. Calcd for $C_{34}H_{42}N_2O_3S_2$: C, 69.12; H, 7.16; N, 4.74. Found: C, 69.09; H, 7.21; N, 4.79.

2-oxo-2-((*3E,5E*)-4-oxo-3,5-bis(4-(trifluoromethyl)benzylidene) piperidin-1-yl)ethylmorpholine-4-carbodithioate (4v):
Yield:74% (pale yellow solid); mp 224-225°C; IR ($CHCl_3$, cm^{-1}): 2866, 2365, 1647, 1615, 1458, 1425, 1328, 1270, 1119, 849; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.70 (t, 4H, *J* = 5.1Hz), 3.91 (brs, 2H), 4.17 (s, 4H), 4.91 (s, 2H), 4.94 (s, 2H), 7.53-7.57 (m, 4H), 7.69 (d, 2H, *J* = 7.3Hz), 7.73 (d, 2H, *J* = 8.0Hz), 7.85 (s, 1H), 7.89 (s, 1H); TOF-MS *m/z* 637.1133 $[M+23]^+$, Anal. Calcd for $C_{28}H_{24}F_6N_2O_3S_2$: C, 54.72; H, 3.94; N, 4.56. Found: C, 54.79; H, 3.89; N, 4.63.

In vitro cytotoxicity:

HCT116 (Colon carcinoma cells) and KBM5 (chronic myeloid leukemia cells) were used for anticancer assay. KBM5 cells were maintained in RPMI-1640,1X (Mediatech Inc., Manassas, USA). DMEM (Dulbeco's modification of Eagle's medium) was used for sustaining HCT116 cells. Both the media were supplemented with 10% fetal bovine serum (Atlanta Biologicals), and antibiotic (10,000 I.U/mL). Cultures were maintained in 75 cm^2 flasks in humidified (95% air) incubator at 37ºC with 5% CO_2.

The cytotoxicity induced by the hybrids was measured by the MTT assay.[27]Briefly, KBM5 and HCT116 cells (5,000 cells/well) were incubated in the presence or absence of5 μM concentrationof indicated test sample in a final volume of 0.1 ml for 72 h at 37°C. Thereafter, 0.025 ml of MTT solution (5 mg/ml in PBS) was added to each well. After 2-h incubation at 37°C, 0.1 ml of the extraction buffer (20% SDS, 50% dimethylformamide, pH 4.7) was added. After an overnight incubation at 37°C, the OD at 590 nm were measured using a 96-well multiscannerautoreader (Dynatech MR 5000, Chantilly, VA), with the extraction buffer as a blank. All compounds were tested three times with three replicates.

Assessment of anti-inflammatory potential; electrophoretic mobility shift assay

To assess the anti-inflammatory potential of these hybrids, we have determined the NF-κB activation in these compounds treated KBM5 cells.The nuclei was isolated from treated-, untreated-, and induced-cells and performed electrophoretic mobility shift assay (EMSA) as described previously.[28] In brief, nuclear extracts prepared from cancer cells were incubated with ^{32}P end-labeled 45-mer double-stranded NF-κB oligonucleotide (15 μg of protein with 16 fmol of DNA) from the HIV long terminal repeat (5′-TTGTTACAAGGGACTTTC CGCTG GGGACTTTC CAGGGA GGCGT GG-3′, with NF-κB-binding sites) for 30 min at 37 °C. The resulting protein-DNA complex was separated from free oligonucleotides on 6.6% native polyacrylamide gels. The dried gels

View Article Online
DOI: 10.1039/C4RA03559G Journal Name

were visualized by Phosphor-Imager imaging device (Molecular Dynamics, Sunnyvale, CA), and radioactive bands were quantified using Image Quant software.

Conclusions

In conclusion, we have reported synthesis of twenty two C-5 curcumin-dithiocarbamates based molecular hybrids, and studied their anticancer activity on KBM5 and HCT116 cancer cell lines, and anti-inflammatory potential. The theoretical studies revealed that many of the hybrids have drug score equivalent or better than curcumin. Twenty one compounds exhibited higher cytotoxicity on KBM5 cell line in comparison to curcumin which was used as a reference compound. The trend of activity was same for KBM5 and HCT116 cancer cell lines. However, eighteen hybrids were found more active than curcumin on HCT116 colon cancer cell line and demonstrated higher potential to inhibit NF-κB activation in comparison to curcumin. Further modification of these compounds is under way and results will be published in due course of time.

Acknowledgements:
DSR acknowledge the Council of Scientific and Industrial Research (02(0049)/12/EMR-II), New Delhi, India for financial support. A.A. is thankful to UGC for research fellowship. Authors are thankful to USIC, University of Delhi for spectral data.

Notes and references

[a]Department of Chemistry, University of Delhi, Delhi-110007, India.
[b]Department of Chemistry, H. N. B. Garhwal University (A Central University), Srinagar (Garhwal), Uttarakhand-246174, India.
[c]Cytokine Research Laboratory and Pharmaceutical Development Center, Department ofExperimental Therapeutics, The University of Texas M. D. Anderson Cancer Center,Houston, Texas 77030, USA.
Corresponding author: Tel:+91 11-27662683; E-mail address:dsrawat@chemistry.du.ac.in (DS Rawat) and msmrawat@gmail.com (M.S.M. Rawat) and Tel: 91-11-27662683;

Electronic Supplementary Information (ESI) available: [details of spectral data with ^{1}H and ^{13}C spectra are available]. See DOI: 10.1039/c000000x/

References

1 (a) R.Kuttan, P.Bhanumathy, K.Nirmala,M.C. George, *Cancer Lett*., 1985,**29**, 197; (b) R.Kuttan, P.C.Sudheeran,C.D. Joseph, *Tumori*, 1987, **73**, 29; (c) H. P.Ammon,M. A.Wahl, *Planta Med*., 1991, **57**, 1; (d) H.Hatcher, R.Planalp, J.Cho, F. M.Torti,S. V.Torti, *Cell. Mol. Life Sci*., 2008, **65***,* 1631.

2 (a) B.B.Aggarwal, B.Sung, *Trends Pharmacol. Sci*., 2009, **30**, 85; (b) A.Goel,A.B.Kunnumakkara, B.B.Aggarwal, *Biochem. Pharmacol*., 2008, **75**, 787; (c) A.Goel, B.B. Aggarwal,*Nutr. Cancer*., 2010, **62**, 919; (d)O. P. Sharma, *Biochem. Pharmacol.*,1976, **25**, 1811; (e) A. J.Ruby, G. Kuttan, K. D.Babu, K. N.Rajasekharan,R. Kuttan, *Cancer Lett.* 1995, **94**, 79; (f) Y. Sugiyama,S.Kawakishi, T.Osawa, *Biochem. Pharmacol.*,1996, **52***,* 519; (g) R. C. Srimal, B. N. Dhawan, *J. Pharm.Pharmacol.*, 1973, **25**, 447.

3 (a) C. D.Lao,M. F.Demierre,V. K.Sondak, *Expert Rev. Anticancer Ther.*, 2006, **6**, 1559; (b) C. D.Lao, M. T.Ruffin, D.Normolle, D. D. Heath, S. I.Murray, J. M. Bailey,M. E.Boggs, J.Crowell,C. L.Rock, D. E.Brenner,*BMC Complement Altern. Med.*,2006, **6**, 10; (c) A. L.Cheng, C. H. Hsu, J. K. Lin,M. M.Hsu, Y. F.Ho, T. S.Shen, J. Y.Ko, J. T. Lin, B. R. Lin, W. Ming-Shiang, H. S.Yu, S. H.Jee, G. S.Chen, T. M. Chen, C. A.Chen, M. K.Lai, Y. S. Pu, M. H.Pan, Y. J. Wang, C. C. Tsai, C.Y. Hsieh, *Anticancer Res.*, 2001, **21**, 2895; (d) G.SHoba,; D. Joy, T. Joseph, M. Majeed, R. Rajendran, P. S.Srinivas, *Planta Med*.,1998, **64**, 353.

4 (a) T. N. Shankar, N. V.Shantha, H. P. Ramesh, I. A.Murthy, V. S.Murthy, *Indian J. Exp. Biol.*,1980, **18**, 73; (b) S. Qureshi, A. H. Shah, A. M. Ageel,*Planta Med.*,1992, **58**, 124.

5 P.Anand, A.B.Kunnumakkara,R.A.Newman,B.B. Aggarwal, *Mol. Pharm.,* 2007, **4**, 807.

6 (a) M. J.Rosemond, St.L. John-Williams,T.Yamaguchi, T. Fujishita, J. S. Walsh, *Chem. Biol. Interact*., 2004, **147**, 129; (b) Y. J.Wang,M. H.Pan, A. L.Cheng, L. I.Lin, Y. S.Ho, C. Y.Hsieh, J. K. Lin, J. *Pharm. Biomed. Anal*., 1997, **15**, 1867.

7 S.C. Gupta, S.Prasad,J.H.Kim, S.Patchva,L.J.Webb, I.K.Priyadarsini,B.B.Aggarwal, *Nat Prod Rep.* 2011, **28**, 1937.

8 (a) T. P.Robinson, T. Ehlers, I. V.Hubbard,X.Bai,J. L.Arbiser, D. J.Goldsmith, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 115; (b) H.Ohtsu,Z.Xiao,J.Ishida, M.Nagai,H. K.Wang,, H.Itokawa,*J. Med. Chem.*, 2002, **45**, 5037; (c) A. Thakur, S. Manohar,C. E. Vélez Gerena,B. Zayas,V. Kumar,S. V. Malhotra, D. S Rawat,*Med. Chem.Commun.* DOI: 10.1039/C3MD00399J,2014.

9 G. Liang,L.Shao, Y. Wang, C.Zhao,Y.Chu,J.Xiao, L.Xiaokun, S.Yang,; Y.Zhao, *Bioorg. Med. Chem.*, 2009, **17**, 2623.

10 B. Cvek, Z.T.Dvorak, *Curr. Pharm. Des.*, 2007, **30**, 3155.

11 R. Schreck, K.Alberman, P. A.Baeuerle,*Free Radical Res. Commun*. 1992, **17**, 221.

12 (a) A. Garg, B. B. Aggarwal, *Leukemia*, 2002, **16**, 1053; (b) G. Sethi, B. Sung, B. B. Aggarwal, *Exp. Biol Med*., 2008, **233**, 21.

13 B. Cvek and Z. Dvorak*, Curr Pharm Des.,*2007, **13**, 3155.

14 (a) Y.Qian, G.Y.Ma, Y.Yang, K.Cheng, Q.Z. Zheng, W.J. Mao, L.Shi, J.Zhao, H.L.Zhu,*Bioorg. Med. Chem.*, 2010, **18**, 4310; (b) W.Huang, Y. Ding, Y.Miao,M.Z.Liu,Y. Li,G.F.Yang, *Eur. J. Med. Chem.*, 2009, **44**, 3687.

15 T. Martens, D. Langevin-Bermond, M.B. Fleury,*J Pharm Sci*., 1993, **82**, 379.

16 W.M. Valentine, V. Amarnath, K. Amarnath, F. Rimmele, D.G. Graham, *Chem Res Toxicol*., 1995, **8**, 96.

17 (a) B. Meunier, *Acc Chem Res* 2008, **41**, 69. (b) K. Liu, D. Zhang, J. Chojnacki, Y. Du, H. Fu, S. Grant, S. Zhang, *Org. Biomol. Chem*. 2013, **11**, 4757, (c) M. Decker, *Curr. Med. Chem.* 2011, **18**, 1464. (d) L. F. Tietze, H. P. Bell, S. Chandrasekhar, *Angew Chem Int Ed Engl*, 2003, **42**, 3996.

Journal Name View Article Online DOI: 10.1039/C4RA03655G COMMUNICATION

18 (a) S.Manohar, U.C.Rajesh,S.I.Khan, B.L.Tekwani, D.S.Rawat, *ACS Med. Chem. Lett.*, 2012, **3**, 555; (b) S.Manohar, S.I.Khan, D.S.Rawat, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 322; (c) N.Kumar, S.I.Khan, Beena,G.Rajalakshmi, P.Kumaradhas, D.S.Rawat, *Bioorg. Med. Chem.*, 2009, **17**, 5632; (d) R. Mamgain, R.Singh, D.S.Rawat, *J. Heterocycl. Chem.*, 2009, **46**, 69; (e) S.Manohar, S.I.Khan, D.S.Rawat, *Chem. Biol Drug Des.,* 2011, **78**,124**.**

19 (a) E.R. Hahm, Y. S. Gho, S. Park, C. Park, K.W. Kim, C.H. Yang, *Biochem. Biophys. Res. Commun.*, 2004, 321, 337; (b) D. Simoni, P. Marchetti, V. Carina, M. Rizzi, R. Rondanin, F. P. Invidiata, M. Notarbartolo, R. Baruchello, M. Labbozzetta, A. Alaimo, P. Poma, N.D' Alessandro, *Bioorg. Med. Chem. Lett.*, 2008, 18, 845; (c) Q. Zhang, Y. Zhong, L. N. Yan, X. Sun, T. Gong, Z. R. Zhang, *Bioorg. Med. Chem. Lett.*, 2011, 21,1010; (d) X. Qiu, Z. Liu,, W. Y. Shao, X. Liu, D. P. Jing, Y. J. Yu, S. L. Huang, X. Z. Bu, Z. S. Huang, L. Q. Gu, L. K. An, *Bioorg. Med. Chem.*, 2008, 16, 8035; (e) G. Liang, S. Yang, H. Zhou, L. Shao, K. Huang, J. Xiao, Z. Huang, X. Li, *Eur. J. Med. Chem.*, 2009, 44, 915; (f) A. P. Zambre, V. M. Kulkarni, S. Padhye, S. K. Sandur, B. B. Aggarwal, *Bioorg. Med Chem.*, 2006, 14, 7196.

20 I.Landais, S.Hiddingh, M.McCarroll, C.Yang, A. Sun, M. S. Turker, J.P.Snyder, M. E.Hoatlin, *Mol. Cancer* 2009, **8**, 133.

21 (a) A.Parvez, J.Meshram,; V. Tiwari, J.Sheik,R.Dongre,M. H.Youssoufi, T. B.Hadda, *Eur. J. Med. Chem.*, 2010, **45**, 4370;(b) A.Parvez, J.Meshram,;T.B. Hadda, *Phosphorus, Sulphur Silicon Relat. Elem*., 2010, **185**, 1;

22 www.molinspiration.com.

23 P. Ertl, B.Rohde, P.Selzer, *J. Med. Chem*., 2000, **43**, 3714;D.E. Clark, *J. Pharm. Sci.*, 1999, **88**, 807.

24 C.A.Lipinski, F.Lombardo, B.W.Dominy,P.J.Feeney, *Adv. Drug Deliv. Rev*., 2001, **46**, 3.

25 www.osiris.com.

26 L. Kumar, A. Jain, N. Lal, A. Sarswat, S. Jangir, L. Kumar, V. Singh, P. Shah, S. K. Jain, J. P. Maikhuri, M. I. Siddiqi, G. Gupta, V. L. Sharma, *ACS Med. Chem. Lett*., 2012, 3, 83.

27 V.Haridas, B. G.Darnay, K.Natarajan, R.Heller, B. B.Aggarwal. *J. Immunol.* 1998, **160**, 3152.

28 M.M.Chaturvedi, A.Mukhopadhyay, B.B.Aggarwal, *Enzymol.* 2000, **319**, 585.