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REVIEW ARTICLE

A review on pharmacophoric designs of antiproliferative agents

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Abstract Past few decades have witnessed the dawn of new diseases in which cancer is a major problem and the race ensued to eradicate cancer by charting out various effective therapeutic regimens. Circumventing resistance issues and combating the toxicity and selectivity problems are matter-of-concern in cancer treatment. Persistent failure to ensure complete remission and eradication of cancer instigated the researchers to exploit the strategies of combining pharmacophores as targeted therapeutic agents. Momentous improvement in the pharmacokinetic as well as pharmacodynamic profile resulting in the enhancement of bioavailability was seen with the introduction of these pharmacophores. The scope of molecular hybridization can be clearly exemplified through the US-FDA approved estramustine and others such as CUDC-101, CBLC-137, PLX3397, E-3810, and CUDC-907 that are currently in different phases of clinical trials. This review seeks to highlight and discuss anti-proliferative activity of some important hybrid, dual, and multi-targeted pharmacophores reported to date along with their designs, structure activity relationships, scope, and limitations. Further, an emphasis has been made to summarize US-FDA approved as well as drugs currently undergoing clinical trials of anticancer drug development.

Keywords Cancer · Pharmacophores · Anti-proliferative agents · Dual inhibitors · Multi inhibitors

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Introduction

Cancer is not just a single disease but a group of multiple diseases characterized by inappropriately controlled cell proliferation and replication eventually resulting in disruption of normal physiology, metabolism, or structure (Abogye and Kaliszczak, 2012; Center, 2013; Hainaut and Plymoth, 2013; Hanahan and Weinberg, 2011; Patrick, 2009; Yance, 2010). It is one of the leading diseases claiming numerous lives and consequently responsible for high mortality rates across the globe (Siegel *et al.*, 2012). Although a number of anticancer drugs are known, most of them have failed to yield the desired result. In most cases, the reason for such failure can be attributed to the lack of selectivity (Folger *et al.*, 2011), less efficaciousness, more side effects (Zitvogel *et al.*, 2013), and multi-drug resistance (MDR) (Daniel and Rauch, 2013). Various strategies have been adopted for combating above-mentioned problems via synergistic action and dose reduction involving either combining two pharmacophores, (2) using a dual pharmacophore in a single molecule, or (3) utilizing the multi-targeted pharmacophore approaches (Bolognesi, 2013). Hence, the effectiveness of such a single molecule having more than one pharmacophore in which each pharmacophore possesses a different mode of action is enhanced several folds (Descoteaux *et al.*, 2012; Meunier, 2007; Morphy and Rankovic, 2005). The pharmacophore concept was introduced by Ehrlich (1909) and defined as, 'a molecular framework that carries (*phoros*) the essential features responsible for a drug's (*pharmacon*) biological activity' (Yang, 2010). According to IUPAC, "a pharmacophore is a single molecule containing a group of steric, electrostatic and hydrophobic properties indispensable for supramolecular interactions with a biological receptor in order to modify or inhibit a biological effect" (Dietis *et al.*,

2009; Wermuth *et al.*, 1998). Moreover, it must obey the Lipinski's rule of five (Zhang and Wilkinson, 2007), should be able to achieve stability in the effective conformation, contain functional groups (proton donor/acceptor, hydrophobic parts) in addition to possessing minimum three of pharmacophoric points (Lipinski, 2004; Proudfoot, 2005; Veber *et al.*, 2002).

An exclusive review was put forward about the latest advances in design and development of hybrid anti-proliferative agents by Fortin and Berube. During the course of preparing the present manuscript, reviews by Nepali et al., Bansal et al., and Fortin et al., which covered the approaches and strategies on hybrids drugs were published (Bansal and Silakari, 2014; Fortin and Berube, 2013; Nepali *et al.*, 2014). In 2007, Junior *et al.*, (Viegas-Junior *et al.*, 2007) and Gediya and Njar (Gediya and Njar, 2009) in 2009 also made successful attempts to compile a review on the design of hybrid anticancer agents. The present review is a concerted effort to bring altogether the different types of pharmacophores; dual/multi-targeted in addition to some important hybrid pharmacophores along with their designs, structure activity relationships, scope, and limitations. Further, an emphasis has been made to summarize US-FDA approved as well as drugs currently undergoing clinical trials of anticancer drug development.

Classification

In this review, we have organised the numerous anticancer agents based on the hybrid, dual, and multi-targeted designs employed for the association of the pharmacophores and the sites they target.

Class I: chimeric/hybrid pharmacophore designs
HDAC and c-Src inhibitor
Chlorambucil-testosterone hybrids
Estradiol-chlorambucil hybrids
Isoflavene-propranolol hybrids
Coumarin-stilbene hybrids
Resveratrol-coumarin hybrids
Isatin-benzothiazole hybrids
Class II: dual pharmacophore/inhibitors design
Dual aromatase steroid sulfatase inhibitors
MAHA and SAHA analogs
Isoindolo [2, 1-*a*] quinoxaline inhibitiors
Dual tubulin and Hsp 27 inhibitiors
Dual-acting HDAC-topoisomerase inhibitors
CUDC–907: a dual inhibitor of PI3 K and HDAC
HDAC and RTK dual inhibitors
Dual inhibitors of Raf 1 and JNK 1
Class III: multi-targeted Hybrids in cancer
Multi-targeted pharmacophores based on HDAC inhibitors
CUDC-101

Class I: chimeric/hybrid pharmacophore designs

Hybrid anticancer agents are of great therapeutic interest as they can potentially overcome most of the pharmacokinetic drawbacks encountered when using conventional anticancer drugs (Fortin and Berube, 2013). A chemical entity constituting two structural domains, depicting dissimilar action as an outcome of acting through different modes of actions are called as hybrid drugs, thus indicating that a hybrid molecule acts as two distinct pharmacophores. Hybrid anticancer drugs are designed on the basis of combining a haptophoric moiety or merging two or more drugs (Fortin and Berube, 2013; Hulsman *et al.*, 2007).

Ko et al. recently revealed and synthesized the hybrid histone deacetylase (HDAC) inhibitor and c-Src inhibitor (non-receptor tyrosine kinase) (Ko *et al.*, 2013). c-Src has been known to play a crucial role in regulating a number of cellular processes in cancer (Fan *et al.*, 2013). The lower efficacy of c-Src inhibitors led the researchers to select an HDAC inhibitor which was seen to arrest and consequently result in apoptosis of cancer cells with fewer side effects. Further, an HDAC inhibitor, panobinostat, was found to have action synergistic with c-Src inhibition. The researchers found this chimeric inhibitor of c-Src and HDAC (Fig. 1) to be more efficacious in NCI-60 cell lines. Further, the downregulation of c-Src levels through suppression of Src transcription was observed to be brought about by HDAC inhibitors. Therefore, such a chimeric/hybrid inhibitor proved to be advantageous in which a single molecule could accommodate inhibitors against c-Src kinase as well as HDAC.

Androgens are not only found to be critical for the growth and development of male sexual organ and function but their role have also been highlighted in the prostate cancer (Boorjian and Tindall, 2013). Principal androgen in the blood is the testosterone (Morgentaler, 2006), whereas in the cells most potent androgen is the DHT (Wright *et al.*, 1996). Increased levels of androgen receptor have been observed in prostate cancer cells (Lunardi *et al.*, 2013). Androgen stimulates proliferation of prostate cells which in turn initiates the progression of malignant tumor (Chang *et al.*, 2013; Zhang *et al.*, 2013). In addition, androgens are also involved in hormone-dependent cancer progression which makes the use of androgen deprivation therapy in prostate cancer patients. Chlorambucil is a potent alkylating agent used in cancer chemotherapy but its side effects including bone marrow suppression, anemia, and weak immune system limits its use (Chabner and Longo, 2011; Denny, 2001). Aiming to explore better alternatives led researchers to the synthesis of hybrid pharmacophore of the hormonal drugs like testosterone with chlorambucil (Fig. 2) (Bastien *et al.*, 2013). These exhibited potent

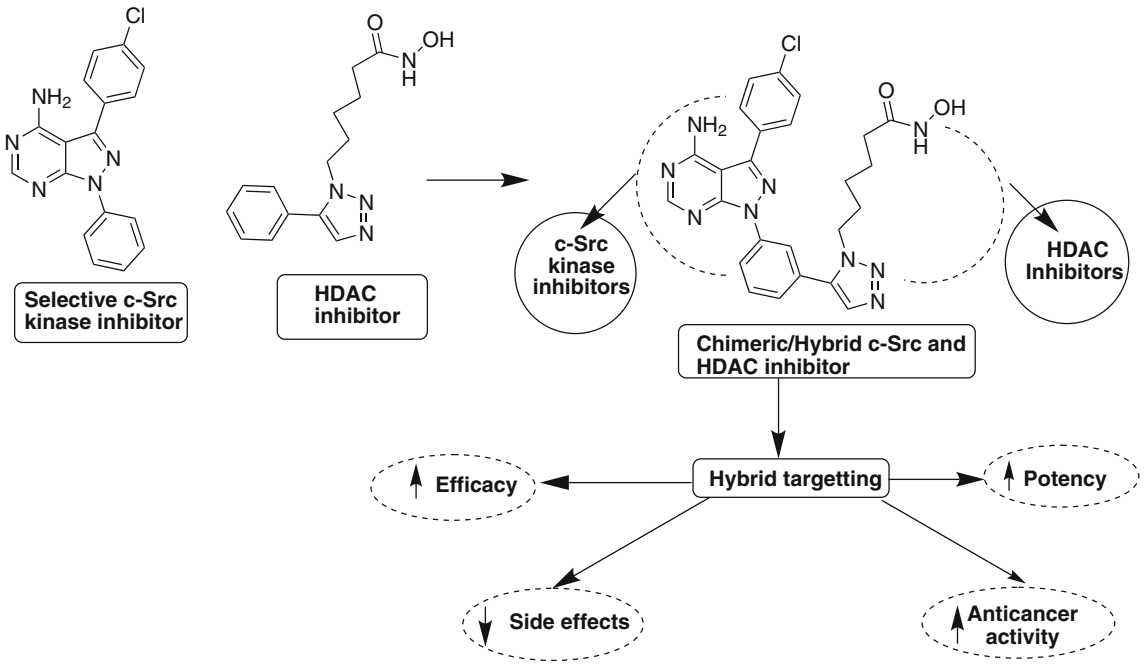
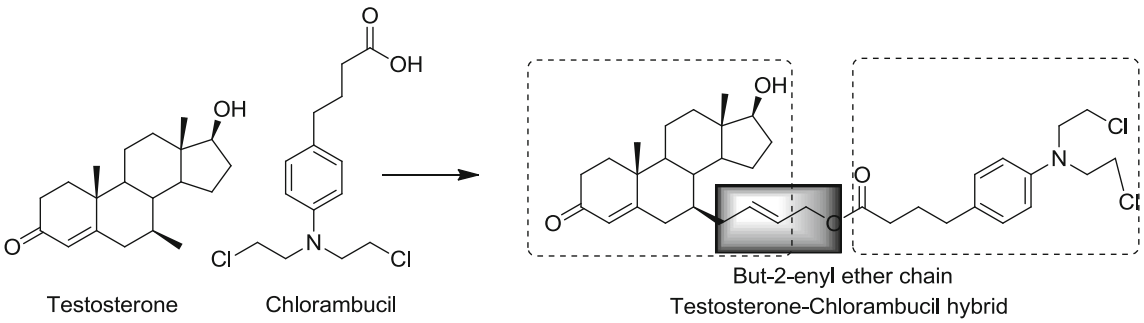


Fig. 1 Hybrid pharmacophore approach for design of HDAC and C-Src inhibitor



S. No.	Cd	LNCaP (AR^+), IC_{50} (μM)	PC3 (AR^-), IC_{50} (μM)
1.	Cyproterone acetate	43 ± 2.5	32.3± 3.7
2.	Chlorambucil	124.3 ± 7.5	131.3 ± 3.1
3.	Testosterone- chlorambucil hybrid	101.0 ± 12.7	–

LNCaP and PC3 are human prostate cancer cell lines.

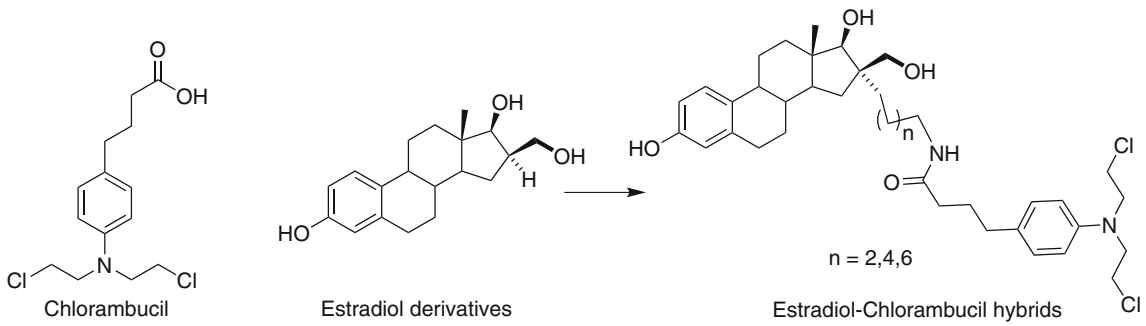
AR^+ = Androgen dependent

AR^- = Androgen independent

Fig. 2 Chlorambucil-testosterone hybrids

inhibitory activity against the hormone-dependent LNCaP (androgen-sensitive human prostate adenocarcinoma cells derived from the left supraclavicular lymph node) cancer cell lines and PC3 cell lines.

Gupta et al., established a series of estradiol-chlorambucil hybrids (Fig. 3) which exhibited moderate to significant cytotoxic activity in hormone-dependent (MCF-7) as well as hormone-independent (MDA-MB-436 and



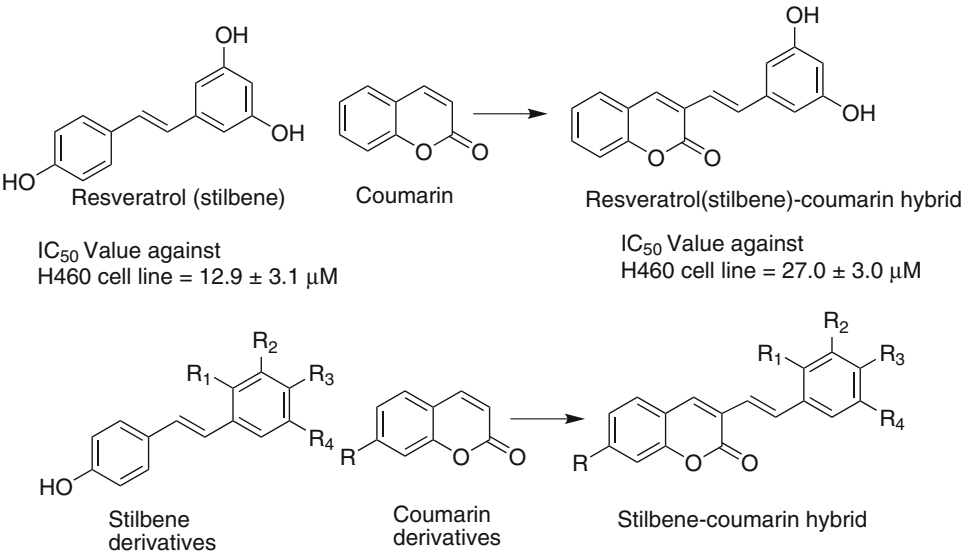
S. No.	Cd	MDA-MB-436, IC_{50} (μM)	MDA-MB-231(ER^-), IC_{50} (μM)
1.	Chlorambucil	> 160	130
2.	Estradiol- chlorambucil hybrid (if n = 2)	40	Inactive

ER^- = Estrogen independent

(MDA-MB-436 and MDA-MB-231) breast cancer cell lines

Fig. 3 Estradiol-chlorambucil hybrids

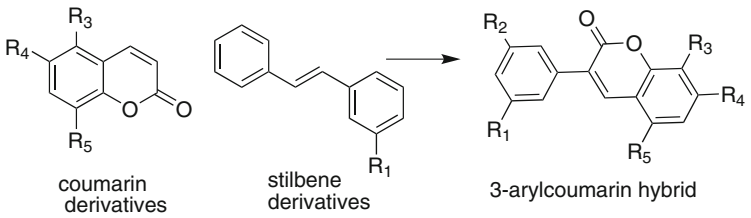
Fig. 4 Resveratrol-coumarin hybrids



Cd	IC_{50} (μM) H460 cancer cell line
R = 7-OCH_3, R_1,R_3 = H, R_2,R_4 = OCH_3	0.45 ± 0.09
R = 7-OCH_3, R_1,R_3 = H, R_2,R_4 = CH_3	0.29 ± 0.08

MDA-MB-231) breast cancer cell lines (Gupta *et al.*, 2010).

Coumarins are a group of naturally occurring compounds (Garcia-Beltran *et al.*, 2014; Singer *et al.*, 2003). They have myriad biological activities including antitumor effects exhibited by the inhibition of cellular proliferation (Ali, 2014; Croteau *et al.*, 2000; Do *et al.*, 2014). Also, stilbenes like resveratrol have been seen to play a crucial role in numerous biological activities especially in cancer (Khan *et al.*, 2013; Kiselev, 2011) and anti-aging by initiating the sirtuin [silent mating type information regulation two homologue (SIRT1)] (Chakraborty, 2013). SIRT1 is an enzyme that deacetylates proteins that help in cellular regulation. The cytotoxic activities exhibited by such compounds may be attributed to their ability to inhibit cell cycle or to induce differentiation and apoptosis. It has also been observed that in physiological quantities, resveratrol possesses the ability to modulate multiple cellular pathways



Cd	IC_{50} KB 468	MCF-7	MCF-7/ADR
1. R_1,R_2 = OCH_3, R_3,R_4 = OCH_3, R_5 = H	5.18	21.43	11.94
2. R_1,R_2 = OCH_3, R_3,R_4 = OAc, R_5 = H	29.15	30.77	39.23
3. R_1,R_2 = OAc, R_3,R_4 = OAc, R_5 = H	9.747	22.94	11.11

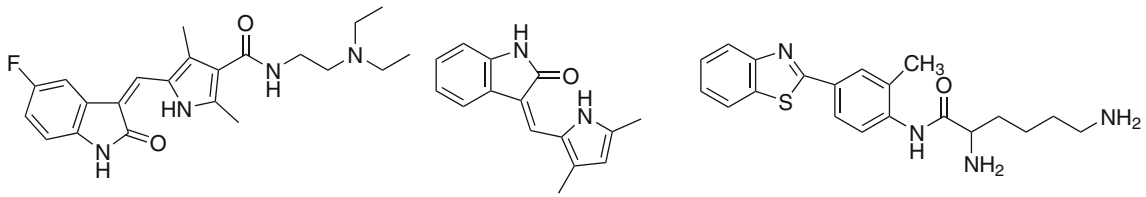


Fig. 6 Isatin analog and benzothiazole analog

critical for tumorigenesis, DNA-synthesis, and various inflammatory responses (Tili and Michaille, 2011). The activities exhibited by these naturally occurring compounds prompted the researchers to synthesize molecules using them in combinations.

Belluti and coworkers synthesized and assessed the hybrid molecules comprising of coumarin scaffold and resveratrol (Fig. 4) for their potential as anti-proliferative agent against H460 lung carcinoma cells (Belluti *et al.*, 2010). The synthesized analogs were also claimed to possess proapoptotic activity which could be attributed to their ability to arrest G2 phase and inhibition of G2/M transition of cell cycle resulting in activation of the apoptotic signals.

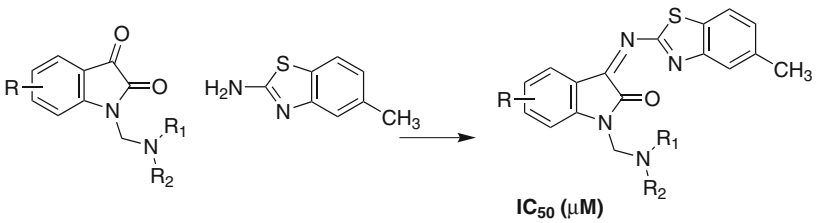
Xiao et al. synthesized novel coumarin-stilbene (Fig. 5) hybrid and tested them for their cytotoxic potential against a panel of cancer cell lines comprising of KB (derived from an epidermal carcinoma of the mouth), MCF-7, and MCF-7/ADR (Xiao *et al.*, 2010).

Isatin (1*H*-Indole-2,3-dione), portraying potent anticancer activity, has been known to be well tolerated by humans (Pandeya *et al.*, 2005). This further highlights the fact that combining together molecules possessing different bioactivities renders the final pharmacophore more active than the combining molecules. Isatin-benzothiazole hybrids (Fig. 6) can be used as a prototype of a new class of anti-breast cancer agents.

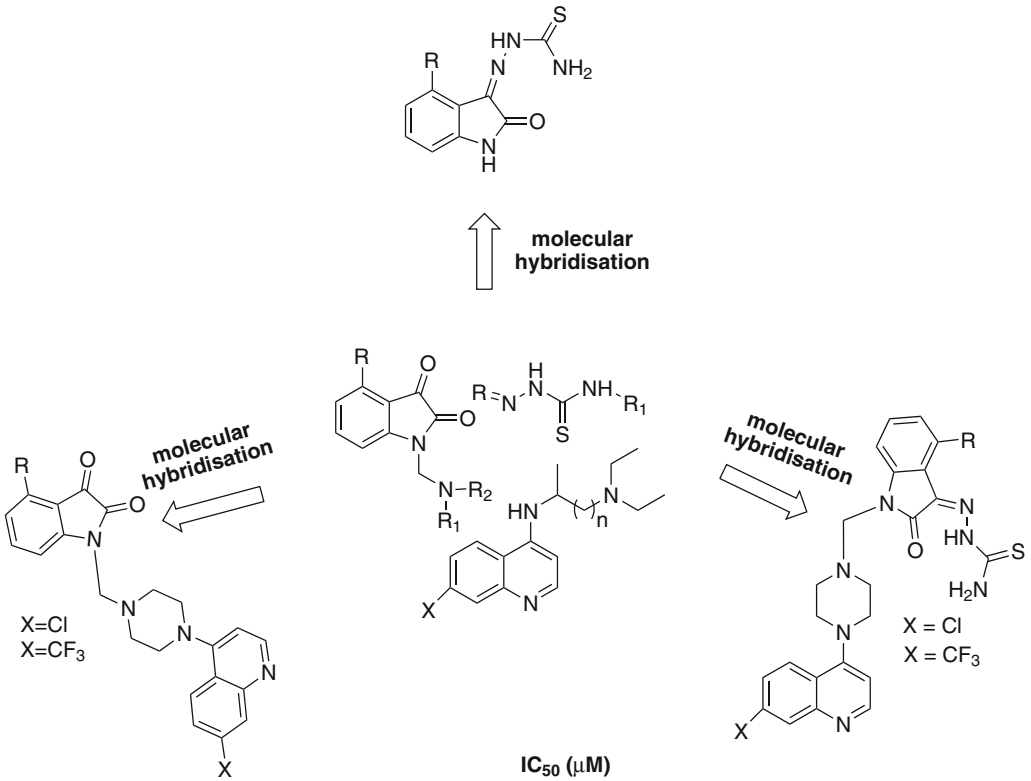
In 2009, Solomon and coworkers designed and synthesized isatin-benzothiazole analogs using the hybrid pharmacophore approach (Fig. 7). The anticancer activity was tested against a panel of human breast cancer cell lines-MDA-MB231, MDA-MB468, and MCF7 of which anticancer activity against the MCF7 cell lines was maximum (Solomon *et al.*, 2009).

In 2010, the same research group synthesized a series of hybrid molecules comprising of 4-piperazinylquinoline based on isatin scaffold i.e., 4-piperazinylquinoline-isatin analogs, 4-piperazinylquinoline-isatin-thiosemicarbazone analogs, and isatin-thiosemicarbazone analogs (Solomon *et al.*, 2010). These analogs were evaluated for their cytotoxic activity against human breast tumor cell lines-MDA-MB468 and MCF7 in addition to two non-cancer breast epithelial cell lines, 184B5 and MCF10A. 4-piperazinylquinoline-isatin analogs and 4-piperazinylquinoline-isatin-thiosemicarbazone analogs exhibited potent cytotoxic activity. They also designed and synthesized 4-piperazinylquinoline and isatin-based hybrid pharmacophore by merging 4-aminoquinolines with isatin moiety via molecular hybridization (Fig. 8). These analogs have shown potent activity against MDA-MB468 and MCF7 cell lines, and these analogs were also virtually screened. IC_{50} value against MDA-MB468 and MCF-7 cancer cell lines has been shown in the range of 10.34–40.36 μM (Solomon *et al.*, 2010).

Fig. 7 Design of isatin-benzothiazole analogs



Cd	IC_{50} (μM) MDA-MB 468	MCF-7	MCF-10A
1. R = H, R_1 R_2 = $(CH_3)_2$	35.45 ± 0.75	29.45 ± 0.23	13.41 ± 0.11
2. R = Cl, R_1 R_2 = Piperdinyl	19.78 ± 0.21	30.94 ± 0.40	30.15 ± 0.44
3. R = H, R_1 R_2 = Morpholinyl	17.61 ± 0.19	14.56 ± 0.21	38.49 ± 0.65
4. Cisplatin (Standard)	31.02 ± 0.45	25.77 ± 0.38	51.51 ± 0.65

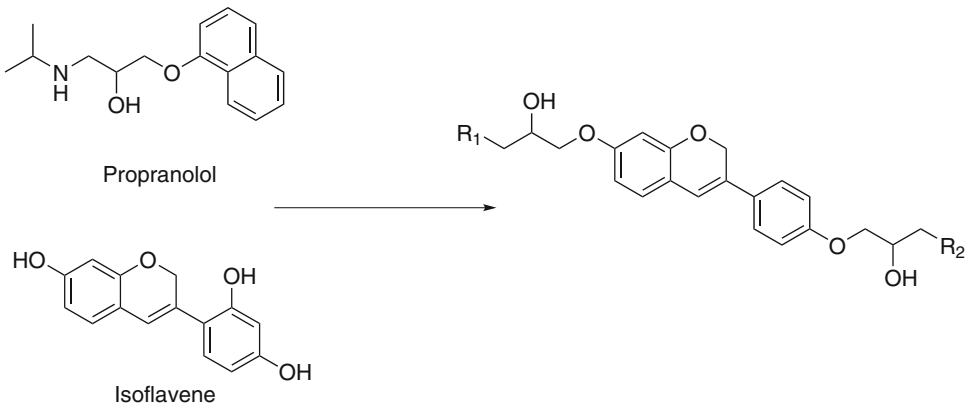


Cd	IC_{50} (μM) MDA-MB 468	MCF-7	MCF-10A
1. X = Cl, R = 4-Br	15.88 ± 0.15	15.12 ± 0.34	65.62 ± 1.68
2. X = CF_3, R = 4-Cl	23.04 ± 0.86	21.56 ± 0.69	51.73 ± 1.02
3. Cisplatin (Standard)	31.02 ± 0.45	25.77 ± 0.38	51.51 ± 0.65

Fig. 8 Design of hybrid compounds using molecular hybridization in 4-aminoquinolines

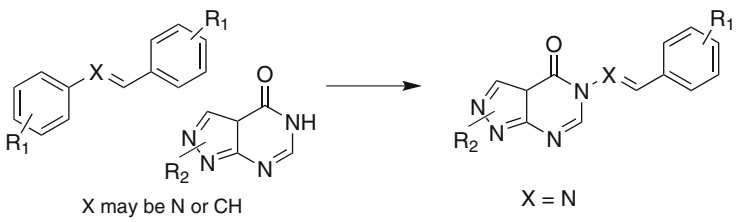
Isoflavones, naturally present in soy products, have been observed to reduce the prevalence of cancer especially that of breast and lung cancer (Mahoney *et al.*, 2012; Messina and Barnes, 1991). Numerous bioactivities exhibited by natural isoflavones led to synthesis of their analogs with increased potency and enhanced bioavailability. Further, the synthetic isoflavene derivatives were also seen to exhibit significant anti-proliferative activity essentially against drug resistant ovarian cancer and prostate cancer at clinical level (Polynesia, 2012). Recently, non-selective β-blocker, propranolol commonly indicated in the treatment of cardiovascular diseases has been shown to enhance the anti-angiogenic and anti-proliferative properties (Ji *et al.*, 2012). Yee et al., explored isoflavene-propranolol hybrids and also evaluated them for their viability assays against SHEP neuroblastoma and MDA-MB-231 breast adenocarcinoma cell lines (Fig. 9). In addition,

Fig. 9 Design of isoflavene-propranolol hybrids



Cd	$IC_{50}(\mu M)$		
	HMEC - 1	MDA-MB 231	SHEP
1. R_1, R_2 = n-hexylamine	0.7	2.9	3.0
2. R_1, R_2 = Ethylamine	2.7	2.0	2.8
3. Isoflavene	4.1	62.9	8.0
4. Propranolol	149	205	109

Fig. 10 Design for pyrazolopyrimidinones and imine hybrids



Cd	$IC_{50}(\mu M)$		
	PC-3	MCF-7	H-460
R_1 = H, R_2 = OMe	17.6	18.1	35.7
R_1 = Cl, R_2 = H	18.1	20.1	39.8

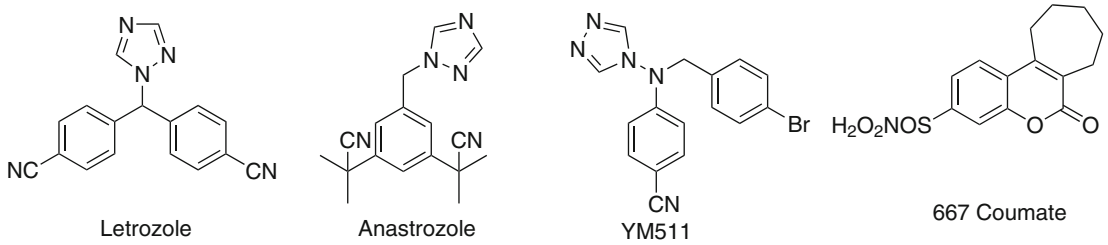


Fig. 11 Aromatase inhibitor and the STS inhibitor

hybrids were also seen to exhibit anti-angiogenic and anti-proliferative activities against human microvascular endothelial cell lines (HMEC-1). The potency of the final hybrid molecules was found to be superior as compared to individual compounds (Yee *et al.*, 2013).

Recently, our research group synthesized and evaluated a series of hybrids of pyrazolopyrimidinones and imine against a panel of cancer cell lines. The compounds were found to inhibit cell growth at G2/M phase of the cell cycle through multiple mechanisms involving DNA damage, free radical scavenging, and topoisomerase-II inhibition (Fig. 10) (Baviskar *et al.*, 2013).

Class II: dual pharmacophore/inhibitors design

Dual pharmacophore/inhibitors are a group of compounds comprising of one structural domain exhibiting dual action by acting through different modes of action but inhibiting

both targets simultaneously (Park *et al.*, 2008; Woo *et al.*, 2003; Wood *et al.*, 2005).

Aromatase inhibitors are mainly indicated in hormone-dependent breast cancer (HDBC). These were developed to inhibit the catalytic action of aromatase enzyme known to play a crucial role in the biosynthesis of estrogens involved in the conversion of androgens to estrogens (Amaral *et al.*, 2013; Bachelot *et al.*, 2012). Clinical application of these aromatase inhibitors for treating advanced

stages of HDBC is well founded, especially in suppressing estradiol levels in plasma to virtually undetectable concentrations (Hanamura *et al.*, 2013). This reduction in the estradiol levels brought about by the aromatase inhibitors can be further accentuated by combining them with steroid sulfatase (STS) inhibitors (Fig. 11) (Nussbaumer and Billich, 2004; Suzuki *et al.*, 2003). STS plays a central role in catalyzing the hydrolysis of steroid sulfates which is mainly responsible for estrogens in cancer and also brings about estrogenic stimulation of hormone-dependent breast tumors as a consequence of modulation of the 5-androstene-3α-17β-diol production (Stanway *et al.*, 2007). Woo and research team merged aromatase inhibitor with STS inhibitor that led to improve the response of hormone-dependent breast tumors to endocrine therapy. This was the result of inhibition of the formation of estrone-3-sulfate in addition to other steroids. Design and synthesis of the dual aromatase steroid sulfatase inhibitor (DASI), possessing

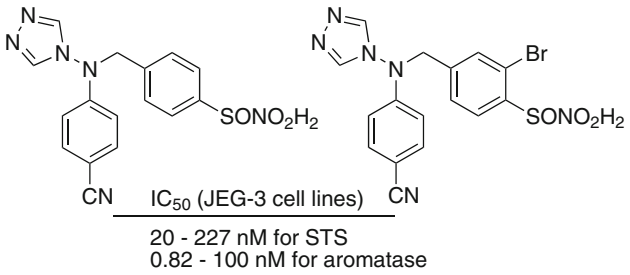
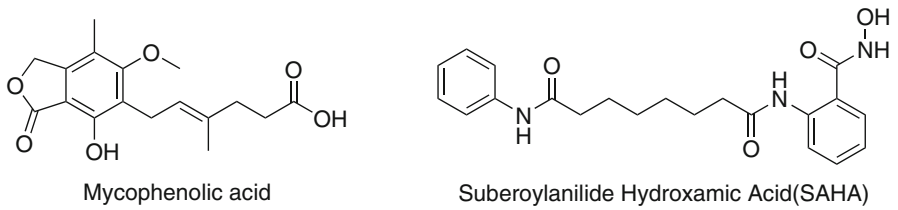


Fig. 12 Dual inhibitor sulphamoylated YM511-derived DASIs

Fig. 13 Mycophenolic acid and suberoylanilide hydroxamic Acid (SAHA)



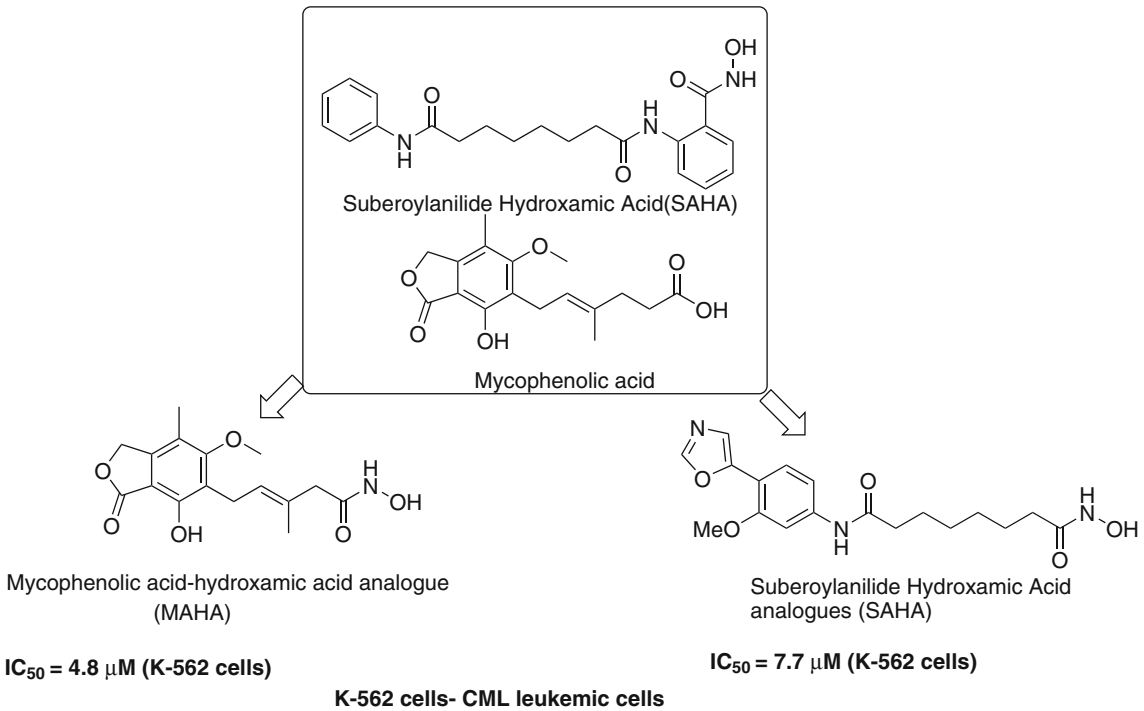
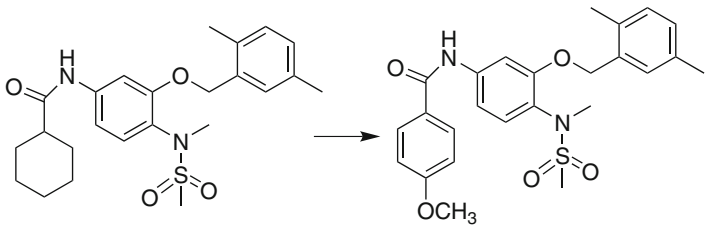


Fig. 14 Mycophenolic acid-hydroxamic acid (MAHA) and suberoylanilide hydroxamic acid analog (SAHA)

Fig. 15 Dual tubulin and Hsp 27 inhibition



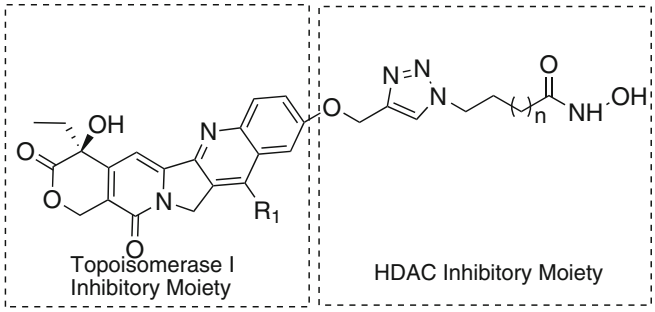
Ist generation

Hsp27 inhibition
IC_{50} = 3 μM against SKBR-3 cells
no tubulin inhibition

2nd generation

IC_{50} = 0.1 μM against SKBR-3 cells
dual tubulin and Hsp 27 inhibition

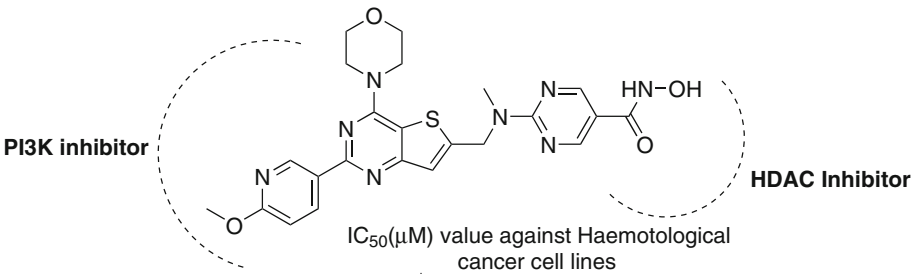
Fig. 16 Dual-acting HDAC-topoisomerase inhibitors



Cd	IC_{50} (μM) DU-145
1. $R_1 = CH_2CH_3$, n=3	2.05
2. R_1 = H, n=2	2.50
3. SN-38 (Standard HDAC inhibitor)	0.11
4. SAHA	2.11

DU-145 (Prostate cancer cells) by MTS

Fig. 17 CUDC–907: a dual inhibitor of PI3 K and HDAC

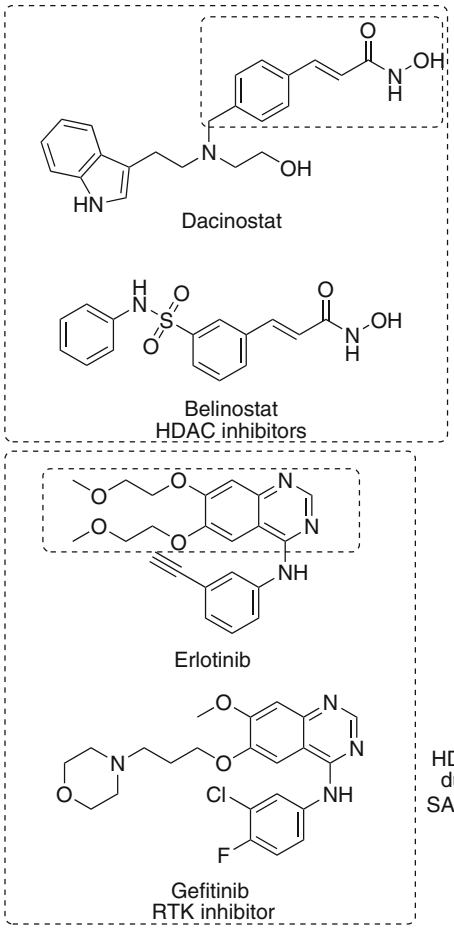


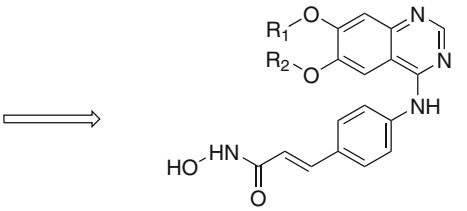
IC_{50}(μM) value against Haemotological cancer cell lines

Cancer type	Cell lines	CUDC-907	SAHA
1. B-cell lymphoma	DOHH2 (foll-icular)	0.001	0.57
	Raji (Burkitt)	0.009	3.56
2. Myeloma	OPM-2	0.001	0.43
	ARH-77	0.005	1.42

the ability to inhibit both the enzymes, resulted in the synthesis of YM511 (Woo *et al.*, 2007, 2003).

Docking, homology modeling, and SAR of DASIs and their corresponding parent phenolic compounds were performed and analyzed by Favia and co researchers (Favia *et al.*, 2006). In addition, DASIs was also docked, by Hernandez-Guzman et al. into the crystal structure of STS to gain insight into the interactions of the





Invitro- HDAC Inhibition IC_{50} (μM)

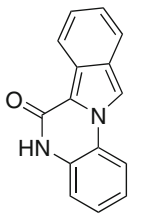
	HDAC-1	HDAC-3	HDAC-6
HDAC and RTK dual inhibitors	0.16 ± 0.02	0.18 ± 0.05	0.56 ± 0.06
SAHA (Standard)	0.25 ± 0.04	0.17 ± 0.02	0.23 ± 0.6

HDAC-1, HDAC-3 and HDAC-6 -NCI-60 cancer cell lines

Invitro- RTK Inhibition IC_{50} (μM)

	EGFR % inhibition	HER-2 % inhibition
HDAC and RTK dual inhibitors	6.1	18.2
Lapatinib (Standard)	92.7	92.0

Fig. 18 HDAC and RTK dual inhibitors



IC_{50} (μM)

PC3	A-549	MCF-7	MCF-7 ^{MDR}
5.59	5.76	0.17 ± 0.02	0.15 ± 0.02

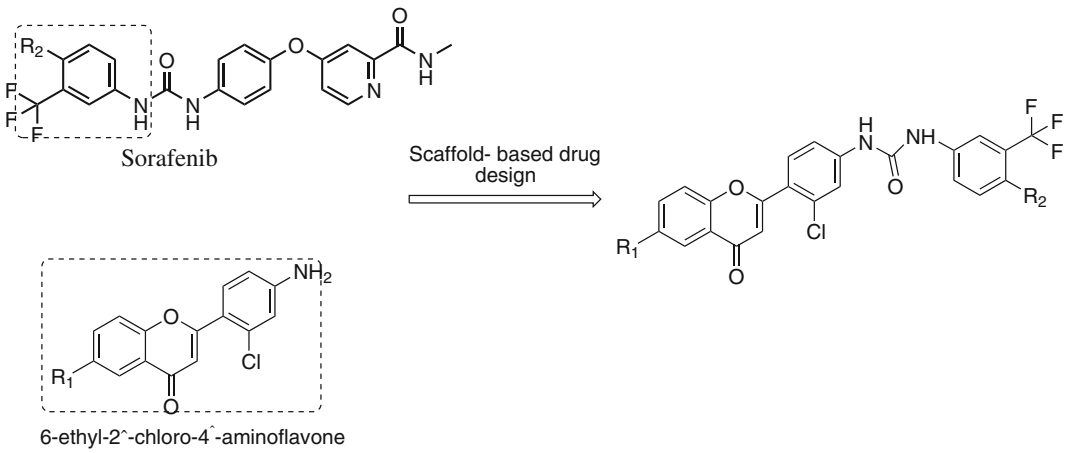
MDR = Multidrug resistant

Fig. 19 Isoindolo [2, 1-*a*] quinoxaline

compound with the enzyme (Hernandez-Guzman *et al.*, 2003).

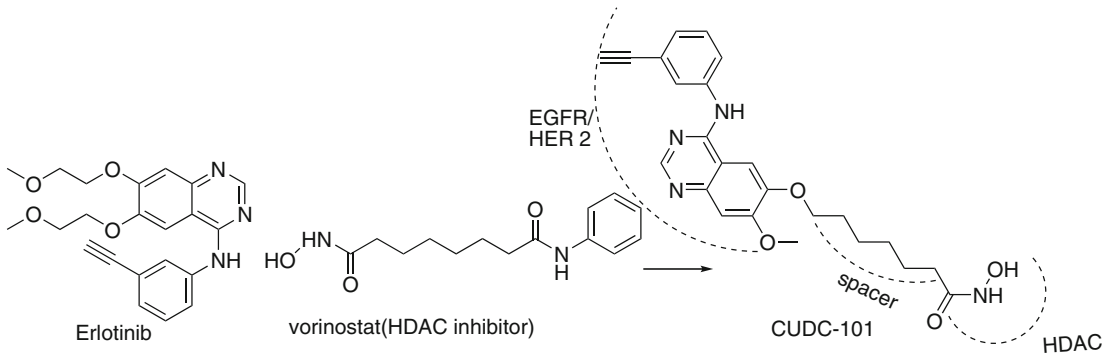
Wood and research group proposed and synthesized new structural class of DASI obtained by introducing sulphamate moiety as the pharmacophore (Fig 12) (Wood *et al.*, 2005).

Mycophenolic acid (MPA) (Fig 13) is an inhibitor of Inosine-5′-monophosphate dehydrogenase (IMPDH), employed largely in transplantation (Fukuda *et al.*, 2011; Glander *et al.*, 2012; Sintchak and Nimmesgern, 2000). This has recently shown to play a central role in cancer treatment as evident from the clinical trials undergoing in patients suffering from advanced multiple myeloma (Chen and Pankiewicz, 2007; Takebe *et al.*, 2006). Moreover, inhibitors of IMPDH are accredited with significant ability of differentiation and apoptosis (Dun *et al.*, 2013). On the other hand, SAHA (Suberoylanilide hydroxamic acid) (Gupta et al.) is the HDAC inhibitor that has been recently approved for the cutaneous T cell lymphoma treatment (Rangwala *et al.*, 2012; Tan *et al.*, 2010; Thurn *et al.*, 2011). SAHA brings about alteration in gene transcription in addition to exerting antitumor effects as a consequence of apoptosis, differentiation, and inhibition of tumor angiogenesis (Marks, 2010).



Cd	IC_{50}		
	HEPG2	A-549	QSG7701
1. R_1 = 6-CH_2CH_3, R_2 = 4-Cl	9.7	14.0	48.9
2. R_1 = H, R_2 = 3-Cl	8.3	> 50	23.7
3. Sorafenib	5.0	4.5	4.2

Fig. 20 Dual inhibitors of Raf 1 and JNK 1



Cancer type	Cell line	IC_{50} (μM) value			
		Erlotinib (E)	Vorinostat (V)	V + E	CUDC-101
NSCLC	H-1975	> 10	4.70	4.00	0.50
NSCLC	H-1703	9.00	4.70	1.80	0.20
Breast	MCF-7	> 20	2.80	2.70	0.55
Prostate	LnCap	18.35	0.58	0.15	0.26

Fig. 21 Generation of CUDC-101

Chen and coworkers carried out the synthesis of compounds exhibiting inhibition against both IMPDH and HDAC (Chen *et al.*, 2013). Merging of MPA to SAHA resulted in the formation of mycophenolic hydroxamic acid which was observed to inhibit both IMPDH and HDAC. SAHA was further modified with moieties that could better interact with IMPDH, thus resulting in compound that could inhibit both IMPDH and HDAC to the same extent as above. Analogs of both MAHA and SAHA were seen to exhibit commendable activity as an anti-proliferative agent in addition to their potential to act as differentiation inducers (Fig 14).

In 2013, Su et al. evidenced that heat shock protein 27 (Hsp 27) and tubulin inhibitors brought about an increased expression of responses such as stress (Su *et al.*, 2013) using SAR. Hsp 27 helps in the survival and inhibits caspase 3 activity through interaction with the pro-caspase 3, thus exhibiting anti-apoptotic activity. Although, it has a molecular weight of 27 kDa, it can form oligomeric complexes. Hsp27 overexpression results in hyperactive chaperone activity which contributes to drug resistance in cancer chemotherapy (Fig 15).

Topoisomerase-I enzyme is known to play a central role in relieving the torsional strain on DNA by cutting one

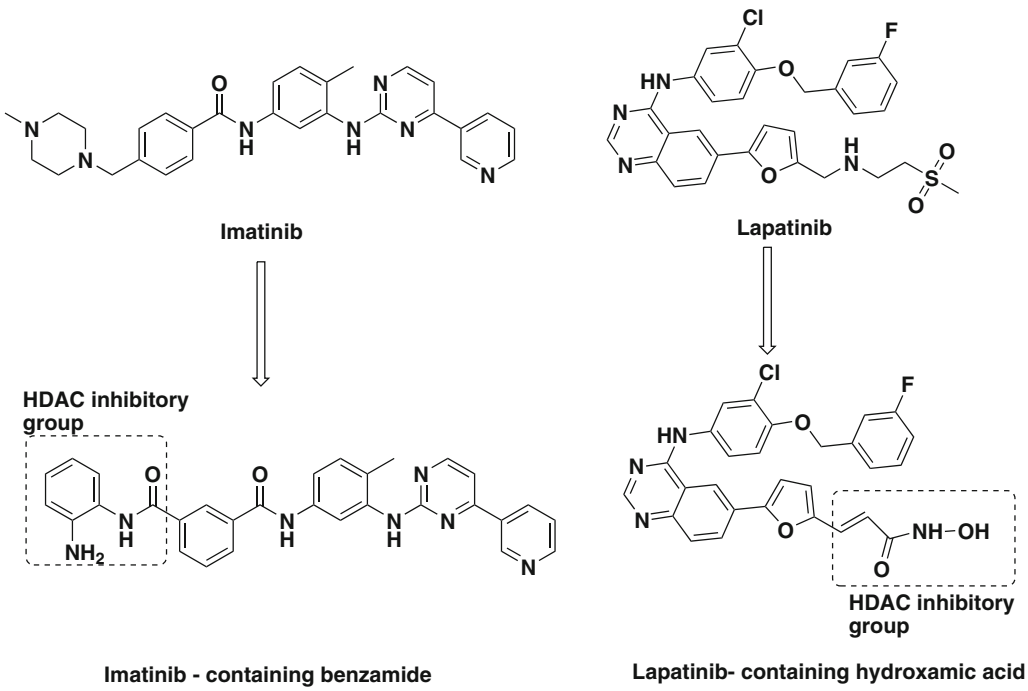


Fig. 22 Multi-targeted pharmacophores based on HDAC inhibitors

Table 1 Drugs in clinical trials

S. No.	Drugs	Phase	Company
1	Estramustine	FDA approved	Pharmacia and Upjohn Company
2	CUDC-907	Phase I	Curis, Inc
3	E-3810	Phase I/II	Ethical Oncology Science (Pastor *et al.*, 2012)
4	CUDC-101	Phase I	Curis, Inc
5	PLX3397	Phase I/II	Plexxicon
6	Curaxin	Phase I	Cleveland BioLabs Inc.

strand of the DNA double helix and passing one strand over the other (Wang, 1996). Inhibitors of topoisomerases bring about DNA strand breaks, cell cycle arrest, and apoptosis, all of which greatly impede replication, one of the important characteristic of cancer, in addition to these activities that would hinder the cell proliferation in cancer. Research supported the fact that topoisomerase I and HDAC inhibitors act synergistically promoting apoptosis in the cancer cells (Johnson *et al.*, 2001; Marchion *et al.*, 2004; Tsai *et al.*, 2000) and both these inhibitors have been found to be concentrated in the nucleus to a higher therapeutic level (Chauhan and Kumar, 2013; Guerrant *et al.*, 2012). On the similar lines, Guerrant et al. evaluated and synthesized dual inhibitors (Fig. 16) by merging together the compounds that possessed the ability to inhibit HDAC as well as topoisomerase I.

Further, Aboagye et al. described that CUDC-907 (*N*-Hydroxy-2-[[[2-(6-methoxy-3-pyridinyl)-4-(4-morpholinyl) thieno [3, 2-*d*] pyrimidin-6-yl] methyl] methylamino]-pyrimidinecarboxamide) (Fig. 17) has the ability to target both PI3 k and HDAC simultaneously.(Abogye and Kaliszczak, 2012) (Delcuve *et al.*, 2013; Flinn *et al.*, 2013; Pursell *et al.*, 2013).

In 2013, Zhang et al. accounted the synthesis and evaluation of compounds that could inhibit both HDAC and receptor tyrosine kinases (RTK) (Zhang *et al.*, 2013). RTK inhibitors (human epidermal growth factor receptor (HER) family) which includes erlotinib, gefitinib, and lapatinib consists of quinazoline moiety and used in treatment of solid tumor cancers (Fig. 18). The combination of the compounds belonging to the two classes resulted in growth arrest, differentiation, and apoptosis in cancer cells.

Diana and group synthesized and evaluated the isoindolo [2, 1-*a*]quinoxaline (Fig. 19) derivatives for their ability to bring about dual inhibition of tubulin polymerisation and topoisomerase (Diana *et al.*, 2008). They successfully merged polycyclic nitrogen heterocycles with quinoxalines. Polycyclic nitrogen heterocycles such as anthracyclins, camptothecin, and amsacrine possessing planar structures have proven to be good pharmacophores owing to their ability to intercalate between the base-pairs of the double stranded DNA. These drugs form ternary complex between the drug, DNA, and enzyme and inhibit the activity of topoisomerase I and II. A large number of quinoxalines and quinoxalinones are reported to act either

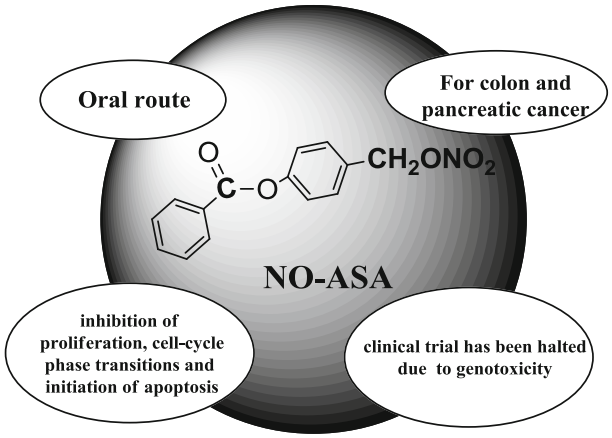


Fig. 23 NO-ASA hybrid

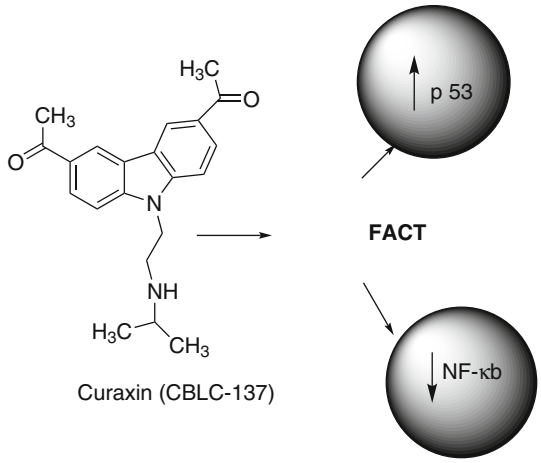


Fig. 24 Multi-targeted curaxin inhibitor

by inhibiting P-glycoprotein or the topoisomerases. The isoindoloquinoxalines displayed inhibitory potential in the micromolar to nanomolar range against a group of 60 human cancer cell lines. Further, polymerization assay carried out in in vitro revealed their microtubule depolymerizing ability.

Jin and coworkers employed the scaffold-based drug design (Fig. 20) for the production of dual inhibitors of Raf1 and JNK1 kinases as anticancer agents. Ability of the flavone scaffold to bring about the inhibition of MAPKs led to the synthesis of compound 6-ethyl-2′-chloro-4-aminoflavone. This compound was assessed as a low-affinity scaffold and was seen to inhibit 14 % p38-alpha activity at 100 mM (Merkel *et al.*, 2013) and 27 % Raf1 activity at 50 mM (Merkel *et al.*, 2013). Using this scaffold, the compounds were generated by merging 6-ethyl-2′-chloro-4′-aminoflavones and diphenylurea derivative. The synthesized compounds were evaluated for their activity against hepatocellular carcinoma. In addition, docking studies performed revealed their binding sites at Raf1 and JNK 1. The synthesized compounds were also found to be less toxic against normal liver cell-lines QSG7701 and HL7702 (Jin *et al.*, 2013).

Class III: multi-targeted hybrids in cancer

The design of multi-targeted hybrids involves linking of pharmacophores possessing different modes of action supported by in silico approaches. This involves the development of rational methods which combine the different structural features from ligands to produce multi-targeted hybrids (Ai *et al.*, 2012; Wang *et al.*, 2013). The multitargeting approach also involves merging of different inhibitors that target a single specific target which could offer a successful means to treat certain diseases such as cancer, type 2 diabetes mellitus, and viral as well bacterial infections (Melisi *et al.*, 2013).

Cai et al. presented the synthesis of series of novel CUDC-101(Jin *et al.*, 2013; Zhang *et al.*, 2014) compound (Fig. 21) and in the process identified multi-targeted hybrid 7-(4-(3-ethynylphenylamino)-7-methoxy quinazolin-6-yloxy)-*N*-hydroxyheptan-amide as a potential drug candidate. CUDC-101 possessed the potential to inhibit simultaneously three different targets comprising of HDAC, EGFR, and HER2. The synthesis involved the introduction of a HDACI moiety into the pharmacophore of the epidermal growth factor receptor (EGFR) and HER2 inhibitors. The results obtained as a consequence of the biological screening suggested that a single compound CUDC-101 shows promising in vitro inhibitory activity against HDAC, EGFR, and HER2 with their corresponding IC_{50} values in nanomolar ranges. Further this compound was observed to exhibit anti-proliferative activity to an extent greater than most of the HDACIs, in vitro. In vivo, CUDC-101 exhibited tumor growth inhibition in various cancer xenograft models such as non-small cell lung cancer (NSCLC), liver, breast, head, and neck, colon, and pancreatic cancers. Phase I study of CUDC-101 has recently been completed in patients with refractory solid tumors (Cai *et al.*, 2010; Lai *et al.*, 2010).

Mahboobi and coresearchers manifested a novel multi-targeting strategy in which the structural features of the Abl, PDGFR-β, and c-Kit inhibitors imatinib were combined with the HDAC inhibitory compounds. This resulted in synergistic inhibition of the different therapeutic targets as well as overcame resistance to imatinib. In addition, this group also reported a similar strategy which involved the merging of EGFR/HER2 kinase inhibitors with the inhibitors of HDAC class 1 or 2 enzymes. In the pursuit to achieve this, a lapatinib scaffold was linked to an HDAC inhibitory head group. This merging resulted in the anti-proliferative activity and proapoptotic, transcriptional

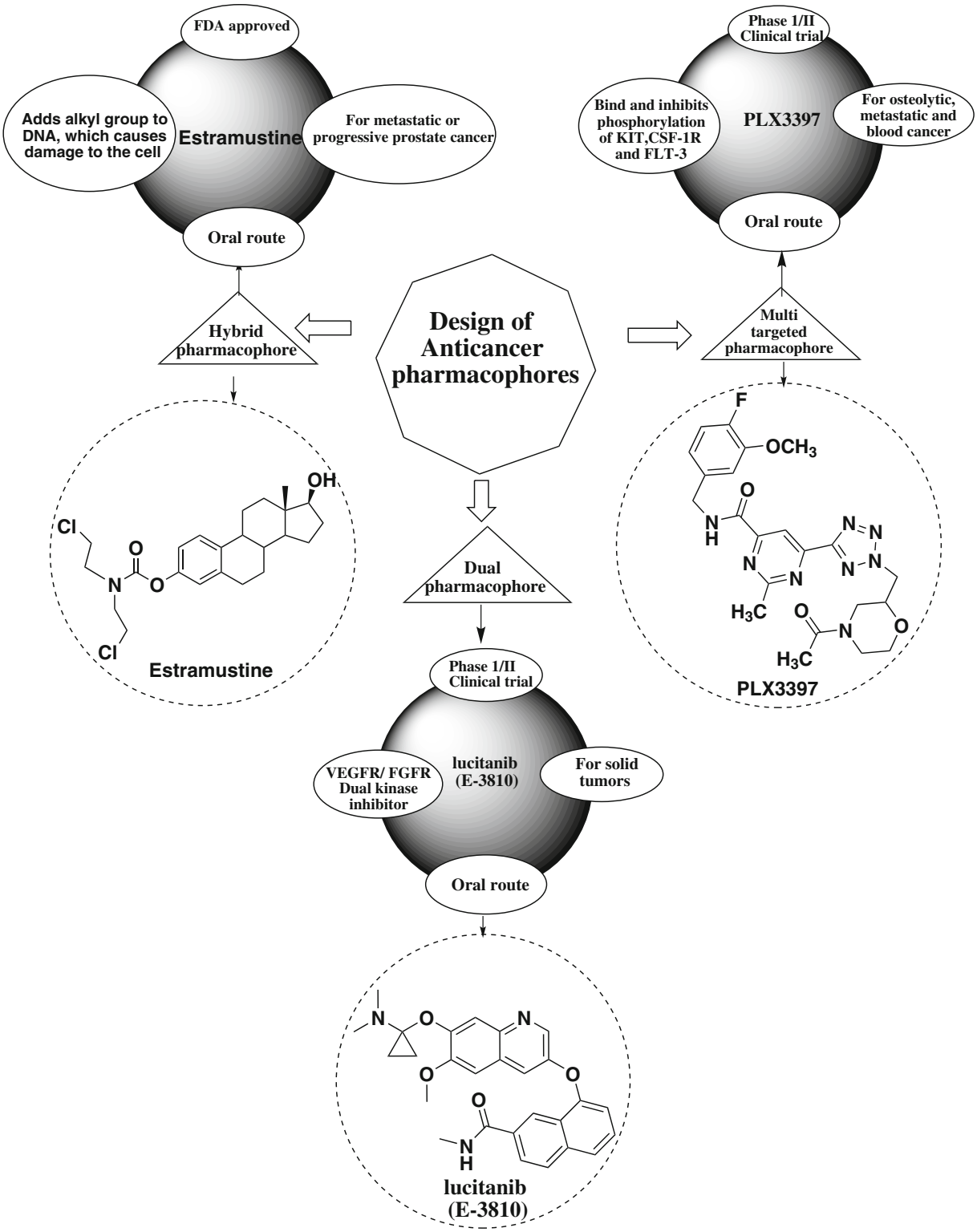


Fig. 25 Anticancer pharmacophores in clinical trials

reprogramming activity. Thus, hydroxamic acid and benzamide moieties were merged with 4-arylquinazoline core structure of lapatinib (Fig. 22) (Mahboobi *et al.*, 2010).

Clinical trials

The above-mentioned approaches have become an area of interest as a number of agents of hybrid, dual, and multi-

targeted pharmacophores are under clinical trials (Table 1). CUDC-907, a dual inhibitor of PI3 k and HDAC that has been discussed above is in phase II clinical trials for non-Hodgkin's lymphoma and multiple myeloma. Further, the studies are conducted on its safety, toxicity, ADME, and biomarker activity. Other drugs in clinical trials are CUDC-101, PLX3397, and E3810. NO-ASA is a chimeric inhibitor consisting of NO and acetylsalicylic acid and had been proposed for chronic lymphocytic leukemia (CLL). Further, it has been studied for its use against prostate and colon cancer. The clinical trial has been halted due to genotoxicity caused by its benzyl nitrate metabolite (Fig. 23).

Curaxin is efficiently linked to the heterodimers of protein i.e., facilitating chromatin transcription (FACT) complex, which results in initiation of p53 activation and NF-κβ inhibition pathways (Fig. 24) ultimately resulting in death of cancer cells (Di Bussolo and Minutolo, 2011; Gasparian *et al.*, 2011).

Metastatic breast cancer Phase I, Phase II clinical trials of PLX3397 for advanced castration-resistant prostate cancer (CRPC) has been completed. Further combination of PLX3397 and paclitaxel is in phase I study for advanced solid tumors and phase 1b study of PLX3397 in combination with vemurafenib in V600-mutated BRAF melanoma is under process (Lin *et al.*, 2013). E-3810 is a multi-targeting inhibitor of VEGF receptor-1,-2, and -3 and fibroblast growth factor receptor-1 tyrosine kinases. This is presently in phase I clinical trials for solid tumors. Further clinical trials on combination of E-3810-paclitaxel have been completed recently (Bello *et al.*, 2013; Molina *et al.*, 2014). A phase I study to evaluate the safety, tolerability and ADME profile of orally administered CUDC-101 in cancer patients has been carried out and currently is in active stage. Further, Phase Ib expansion study for the safety, efficacy, and pharmacokinetics of intravenous CUDC-101 against advanced head and neck, gastric, breast, liver, and NSCLC tumors has been completed (Wang *et al.*, 2013). Estramustine is the hybrid FDA approved drug for the prostate cancer (Fig. 25) (Caffo *et al.*, 2010; Noguchi *et al.*, 2010; Picus *et al.*, 2011; Ravery *et al.*, 2011). This is an antimicrotubular agent and combination of nitrogen mustard and estradiol. It is also named as estradiol 3-[bis(2-chloroethyl)carbamate]-17-(dihydrogen phosphate), disodium salt, monohydrate.

Conclusions

From the literature search, it is anticipated that anti-proliferative compounds emerged through either of the above-mentioned designs acting synergistically at target site(s), with increased selectivity, potency, lesser resistance, and side effects will definitely find their scope at the clinical level. This has been corroborated by the recently FDA approved estramustine and the drugs undergoing clinical trials. However, following issues need to be addressed or rectified when designing the hybrid, dual or multi-target inhibitors:

(a) Physicochemical properties, drug interactions, manufacturing and regulatory challenges may come into picture and limit their uses which may be due to the reasons that these agents owing to their large size have high molecular mass and increased lipophilicity, thus violating Lipinski's rule.
(b) Molecular hybridization sometime fail to maintain balance between potency and safety as seen in case of NO-ASA, a chimeric inhibitor which was proposed for CLL, prostate and colon cancer. But the clinical trials have been halted due to genotoxicity which was also present in parent molecules.
(c) Chemical stability of the compounds should be taken into consideration as they may get cleaved before they reach the site of action.
(d) An understating and knowledge of various pathways, receptors, targets, and mediators involved in cross-talk of cancer cell signaling may definitely support in rationale designing of inhibitors.

Further, a lot of work has been done and is being carried out using hybrids of inhibitors of histone deacetylases, kinases, tubulins, hormones, etc. but other targets like chemokines and cellular pathways involving cross talks appear to be unexplored.

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