DR FEROZ KHAN (Orcid ID : 0000-0002-2704-5925)

Article type : Research Article

An improved synthesis of indanocine and antiproliferative activity of 2-benzyl-indanocine *via* microtubule destabilization

Sadiya Khwaja,[1,3] Kaneez Fatima,[1,3] Divya Mishra,[1] Vineet Babu,[1] Yogesh Kumar,[1] Sumera Banu Malik,[2] Misbah Tabassum,[2] Suaib Luqman,[1,3] Dnyaneshwar U. Bawankule,[1,3] Debabrata Chanda,[1,3] Feroz Khan,[1,3] D. M. Mondhe,[2,3] Arvind S. Negi[1,3*]

[1.] *CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) P.O. CIMAP, Lucknow, 226015, India*

[2.] *CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Canal Road, Jammu-180001, India.*

[3.] *Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, India*

ABSTRACT

Indanocine, a potent anticancer investigational drug of National Cancer Institute-USA has been much discussed in recent years. Present communication aimed at total synthesis of indanocine and its close analogues. Total synthesis was improved by double yields than previously reported yields. Some of the benzylidene and 2-benzyl derivatives with free rotation at C2 position exhibited potential cytotoxicities against various human cancer cell lines. Five such analogues exhibited potential antiproliferative effect against HCT-116 and MIA-PACA-2 cell lines. Benzylindanocine **12i** induced microtubule destabilization by occupying colchicine binding pocket of β-tubulin. It also exhibited antiinflammatory activity by down-regulating IL-6 and TNF-α. In Ehrlich ascites carcinoma model **12i** reduced 78.4% of EAC tumour in Swiss albino mice at 90 mg/kg (*i.p.*) dose. Further, in *in-vivo* safety studies **12i** was found to be safe to rodents up to 1000 mg/kg dose. Concomitant anticancer and antiinflammatory activity of benzylindanocine is distinctive which suggests its further optimization for better efficacy and druggability.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CBDD.13857

Keywords: Indanocine, Anticancer, antitubulin, Ehrlich ascites carcinoma, Acute oral toxicity

……

*Corresponding author: E-mail: arvindcimap@rediffmail.com ; ; Tel: +91-522- 2342676 x 583; Fax: +91-522-2718509;

CIMAP communication No. CIMAP/PUB/2020/Jun/43

1. INTRODUCTION

Worldwide, cancer has caused 9.6 million human deaths which is about 17% of total human deaths. In 2010, total annual economic cost of cancer was US$1.16 trillion [**WHO Keyfacts 2018**]. Researchers are considerably exploring to identify diverse chemotherapeutics to tackle the disease. Modulation of tubulin-microtubule dynamics is a distinctive capability of antimitotic agents. Highly dynamic mitotic spindle microtubules are remarkable therapeutic targets for a group of chemically diverse and clinically successful anticancer drugs. Even with the advent of targeted therapies for cancer, antimitotic agents are some of the most effective drugs in the treatment of solid tumours and hematological malignancies [**Kaul et al. 2019**]. A number of antimitotic agents have successfully been developed as clinical anticancer drugs like paclitaxel, docetaxel, cabazitaxel, ixabepilone, vinorelbine, vinflunine, eribulin mesylate (E7389), ENMD-1198 and STX-140 etc. and many more are under the development of various phases of clinical trials namely epothilone derivatives patupilone (Phase-III), sagopilone (Phase-II), BMS310705, larotaxel (Phase-III), tasetaxel (Phase-II), taxoprexin (Phase-III), xyotax (Phase-III), combretastatin A4 and its phosphate salts (Various phases of clinical trials), dolastatin (Phase-II), cryptophycin52 (Phase-II & Phase-III), halichondrins (phase-I), and noscapine (Phase-II) etc. [**Negi et al. 2015**].

[Fig.1]

Indanocine (**1**), a synthetic indanone is a potent antiproliferative agent developed by NCI, USA. It is a potent tubulin binding drug effective against a number of human cancer cell lines (mean GL_{50}<20 nM) and against multidrug resistant cell lines [**Leoni et al. 2000**]. Studies demonstrated that tumour cells over-expressing 170KD P-glycoprotein resistant to paclitaxel were very much sensitive to indanocine. As a microtubule destabilizer, it binds to colchicine binding site of β-

tubulin [**Das et al. 2009**]. Some other studies demonstrated that indanocine inhibited migration of metastatic cancer cells [**Kapoor and Panda 2012**]. Owing to an important antimitotic anticancer agent several analogues of indanocine have also been prepared in the recent past [**Huang et al. 2012; Tunbridge et al. 2013**].

[Fig.2]

Present communication reports an improved synthesis of indanocine. The process is very efficient and straightforward where new set of reagents have been used for better yields. The overall yield of the process is 26.7% in three synthetic steps which is much better than the previously reported methods [**Shih et al. 2000; Coulup and George 2019**]. Sixteen other analogues of indanocine were also synthesized. Our main aim was to synthesize more flexible analogues of indanocine to have better interaction with tubulin protein. So, we planned to have 2-benzyl analogues of indanocine where 2-benzylidene ring was reduced for more flexibility. All these analogues were evaluated against four human cancer cell lines. Benzylindanocine exhibited potent antiproliferative activity against two human cancer cell lines. Both indanocine and benzylindanocine were extensively evaluated for target studies, druggability, *in-vivo* efficacy and benzylindanocine for safety in Swiss-albino mice.

2. RESULTS

2.1. Chemistry

Synthesis of indanocine

All the previous and present synthetic protocols have been depicted in Scheme 1. Previously, two protocols were reported and both were from the same group of UCSD following identical route of synthesis. Carson *et al.* mentioned only last two steps of protocol in this patent. In the first step 1N NaOH in ethanol was used at 50^{o}C to get nitroindanone derivative **11** in 96% yields from nitro derivative **10**. In the final step, nitro group of **11** was reduced with alkaline sodium bisulphite solution to afford indanocine (**1**) in 18% yield. In this patent, preparation of compound **10** was not mentioned. The overall yield in two steps was 17.3% only [**Carson et al. 2000**]. The second communication by Shih *et al.*, nitration of indanone **9** was done with sodium nitrite in TFA to get nitroindanone **10**. In the next step, 2-benzylidene derivative of nitroindanone (**11**) was prepared by condensation of 3,5-dimethyl-4-hydroxybenzaldehyde using 5% methanesulphonic acid in acetic acid. Reduction of nitro group of **11** was tried with two different reagents i.e. Sodium dithionate or zinc dust in 2% acetic acid to get indanocine (**1**). However, yields of these steps were not mentioned in this communication [**Shih et al. 2000**].

In our strategy, we also followed the identical route of synthesis as done by UCSD group but, using other set of reagents. In the first step, nitration of indanone **9** was done in cold acetic anhydride-nitric acid mixture to get compound **10** in 74% yields. Compound **10** was condensed with 3,5-dimethyl-4-hydroxybenzaldehyde in dioxane-conc. HCl system under reflux conditions to get 2-benzylidenenitroindanone (**11**) in 82% yield. Finally, nitro group of **11i** was reduced under controlled conditions with 10% Pd-C in dry DMF to afford indanocine (**1**) in 44% yield. The overall yield of our process was 26.7% in three steps.

[Scheme 1]

Synthesis of indanocine analogues

Synthetic strategy for preparation of indanocine analogues (**11a-11m**, **12a-12m** & **13n-13p**) was as illustrated in Scheme 2. To get diverse 2-benzylidene derivatives, various substituted benzaldehydes were condensed with 7-nitro-5,6,-dimethoxyindanone-1 (**10**) *via* Claisen Schmidt reaction using different reaction conditions (One alkaline and two acidic conditions) to get 2-benzylideneindanone derivatives (**11a-11l**) in 43-86% yields. Boc-piperazine derivative **11l** was treated with trifluoroacetic acid in dry DCM under cold conditions to afford 2-benzylidene derivative **11m** in 91% yield. All the 2-benzylidene-7-nitro indanone derivatives were reduced with 10% Pd-C-dry DMF at RT to get fully reduced 2-benzyl-7-aminoindanone derivatives **12a-l** in 44-67% yields. But, compounds **12n-12p** underwent partial reductions (Nitro group) only and afforded 7-amino-2-benzylidenes (**13n-13p**). Nitration of 5,6-dimethoxyindanone (**9**) in $NaNO_2$-BF_3-etherate-MeOH system yielded nitration at 2-position and product obtained was rather **14** (aci form) due to strong hydrogen bonding. All products were confirmed by spectroscopy.

[Scheme 2]

2.2. Biological evaluation

Antiproliferative activity

All derivatives of indanocine were evaluated for antiproliferative activity by SRB assay against four human cancer cell lines *viz.* HCT-116 (Colon), MIA PACA-2 (Pancreas), SW 620 (Colon) and A549 (Lung). Initially, compounds were evaluated at two different concentrations of 10 μM and 50 μM. Out of twenty nine analogues fifteen analogues exhibited significant cytotoxicity (≥50% inhibition at 50 μM), While five analogues exhibited potent cytotoxicity (≥50% inhibition at 10μM) (Table 1A). On further evaluation, indanocine (**1**) exhibited IC_{50} of 1.03 μM (HCT-116) and 0.44 μM (MIA PACA-2) while benzylindanocine (**12i**) exhibited IC_{50} of 1.30 μM and 0.58

μM against both the cell lines respectively (Table 1B). However, both the compounds were less active than standard drug combretastatin A4 (IC_{50}<0.1 μM).

[Table 1A & 1B]

For structure activity relationship, we had mainly two series, Series-I as 2-benzylidene-7-nitroindanones (**11a**-**11m**) and Series-II as 2-Benzyl-7-aminoindanones (**12a**-**12m**). Cytotoxicity against MIA PACA-2 cell line (at 50 μM) was taken into consideration as most of the compounds exhibited higher activity against this cell line. In general, Series-II compounds were more active than the Series-I compounds. In Series-I, compound **11a** was without any substitution, introduction of a methoxy group (**11c**) marginally increased the cytotoxicity, cytotoxicity was further increased on introducing second methoxy group (**11f**). But, on introducing third methoxy group (**11h**), activity was decreased drastically. When a fluoro (**11j**) or hydroxy (**11b**) group was introduced in **11a** cytotoxicity was enhanced. Further, demethylation of any methoxy group of **11f**, resulted in decreased cytotoxicity (**11d** and **11e**). Introduction of amino group in compounds (**11k**, **11l**, **11m**) possessed marginally higher activity. In Series-I, 4-hydroxybenzylidene derivative (**11b**) possessed the highest cytotoxicity.

In Series-II (**12a**-**12m**), compound**12a** was 2-benzyl derivative with no substitution. Introduction of one methoxy group (**12c**) significantly reduced cytotoxicity. But subsequent introduction as dimethoxy (**12f**) and trimethoxy (**12h**) groups enhanced activity gradually. Both 4-hydroxy (**12b**) and 4-fluoro (**12j**) derivatives showed less cytotoxicity than **12a**. Subsequent introduction of two methyl groups (**12i**) enhanced drastically, which was the best compound in this series. Both piperazine based benzyl derivatives (**12l** and **12m**) possessed higher activity than **12a**.

Tubulin polymerisation inhibition activity

Tubulin targeting agents disrupt cellular process by modulating tubulin-microtubule dynamics [**Coulup and George 2019**]. Effect of indanocine (**1**) and benzylindanocine (**12i**) on polymerisation process of tubulin was determined by tubulin kinetics experiment. Paclitaxel was used as standard stabilizer and podophyllotoxin as standard destabilizer. Three different concentrations of each test compound were taken. Both the compounds exhibited moderate destabilization of microtubules. Figures 3A and 3B show paclitaxel curves are above the GTP protein clearly exhibiting stabilization effect. Podophyllotoxin treated curves are much below the GTP protein showing strong destabilization effect. Both indanocine (**1**, Fig. 3A) and benzylindanocine (**12i**, Fig. 3B) treated curves are also below the GTP protein curve, distinctly exhibiting destabilization effects. But, their destabilization effects are lower than podophyllotoxin.

Hence, both compound **1** and **12i** exhibit moderate destabilization of tubulin polymerisation process.

[Fig 3A & 3B]

Antiinflammatory activity of 2-benzylindanocine

Tumour microenvironment contains many different inflammatory cells and mediators (Cytokines, chemokines, and prostaglandins) that are over-expressed during the pathogenesis of the disease. Both TNF-α and IL-6 are well established prognostic markers in acute cancer inflammation [**Furman et al. 2019**]. Interestingly, both indanocine (**1**) and benzylindanocine (**12i**) significantly inhibited LPS-stimulated productions of pro-inflammatory cytokines TNF-α and IL-6, thus exhibiting cancer related antiinflammatory activity as well (Table 2). Both indanocine (29.36%) and benzylindanocine (28.38%) showed comparable activity against TNF-α. Benzylindanocine (**12i**, 46.02%)) exhibited higher IL-6 inhibition activity as compared to indanocine (**1**, 41.41%)). However, both the compounds had relatively lower activity than the standard anti-inflammatory clinical drug dexamethasone.

[Table 2]

Molecular docking studies

a) Interaction with target protein β-tubulin

In a comparative molecular docking studies of indanocine (**1**), both 2α (2R) and 2β (2S)-benzyl indanocine (**12i**), podophyllotoxin and colchicine were used as standard destabilizers of β-tubulin. All five ligands occupied same binding pocket of β-tubulin (Table 3). There were six residual amino acids common to all i.e. LEU B:248, ALA B:250, LEU B: 255, ASN B:258, ALA B316 and LYS B:352 indicating a common binding site. 2β-Benzyl derivative of indanocine showed highest binding affinity (-8.1 Kcal/Mol) with β-tubulin. It showed marginally better affinity than indanocine (-8 Kcal/Mol). While, 2α-benzylindanocine had less affinity (-7.9 Kcal/Mol) than the indanocine. However, all these three ligands (**1**, **12i-α** and **12i-β**) possessed higher binding affinity with β-tubulin than the both the standard inhibitors colchicine (-6.2 Kcal/Mol) and podophyllotoxin -7 Kcal/Mol). Further, docking view showed a common site occupied by these ligands. They also exhibited structural similarities as far as chemical space is concerned (Fig. 4).

[Table 3] [Fig. 4]

b) In-silico prediction of ADME properties

Physicochemical properties of drug candidate give an idea about the druggability of molecule. Pharmacokinetic properties of both indanocine (**1**) and 2-benzylindanocine **12i** were calculated

using online SwissADME software. Physicochemical parameters, water solubility, lipophilicity, pharmacokinetics, druglikeness and medicinal chemistry aspects were calculated. Both compounds showed good druggability. All the required parameters were within desired limits. Both did not possess PAINS (Pan-assay interference compounds) structure and did not violate Lipinski's rule. Overall, both compounds possessed satisfactory oral bioavailability and hence druggability. Further, figure 5 exhibits bioavailability radar of both compounds within defined limits of good bioavailability.

[Figure 5]

In-vivo antiproliferative efficacy by Ehrlich Ascites Carcinoma

Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma which is well established model in tumour biology for the study of tumour pathogenesis and development of anticancer agents. Both indanocine (**1**) and 2-benzylindanocine (**12i**) were evaluated for their *in-vivo* anticancer efficacy by EAC in Swiss albino mice. Both indanone derivatives **1** and **12i** exhibited significant antitumour activity (Tables 5a and 5b). However, efficacy of compound **1** was much better than compound **12i**. Indanocine reduced 89.1% of EAC tumour at 30 mg/kg dose (*i.p.*), while 2-benzylindanocine (**12i**) reduced 40.2%, 53.8%, and 78.4% of tumour at 10 mg/kg, 30 mg/kg and 90 mg/kg dose (*i.p.*) respectively. There was no mortality in any of group throughout experimental period. Standard drug, 5-fluorouracil exhibited 97.2% tumour reduction at 20 mg/kg *i.p.* dose.

[Table 5A & 5B]

*In-vivo safety studies of **12i***

Acute oral toxicity specifies various adverse effects aroused on oral administration of single dose of test compound to experimental animal. There were no observational changes throughout the experimental period of **12i** up to dose level of 1000 mg/kg body weight. There were no detrimental effects in body weight of experimental mice. It did not induce any toxic effect in haematological parameters, liver function parameters, kidney function parameters, and lipid parameters of experimental animals (Table 6). However there was slight reduction in WBC count which was statistically in significant. Gross pathological studies (Fig. 6A & 6B) showed no significant changes in the absolute and relative organ weights of vital organs. Furthermore, differential leukocyte count did not show any sign of toxic effects of compound **12i** like invasion

or inflammation (Fig. 7). Overall, 2-benzylindanocine **12i** was safe and well tolerable up to dose level of 1000 mg/kg body weight as a single acute oral dose in Swiss albino mice.

[Table 6]

[Figure 6A, 6B & Figure 7]

3. DISCUSSION

Indanocine is a potent investigational anticancer drug. Our first objective was to develop an improved protocol for its synthesis. We used same reaction route as previously done by Carson group but a new set of reagents. The reagents were more efficient to improve yield of the process by two fold. This group did not mention yield in the first step (Nitration of indanone) but in other steps our overall yield was much better.

Colon and pancreatic cancers are among leading causes of cancer deaths. Globally, both colorectal and pancreatic cancers caused more than 1 million deaths annually. Chemotherapy and surgery are two most common medical remedies to tackle the disease. Chemotherapy is a preferred approach with combination of various anticancer drugs with meager success rate of 64% for five years in colon cancer and only 7% for five years in pancreatic cancer [**Cancer facts and figures 2020**].

2-Benzylindanocine **12i** exhibited significant antimitotic activity. We planned to synthesize more flexible analogue of indanocine *i.e.* 2-benzylindanocine. In *in-vitro* studies, indanocine exhibited better cytotoxicity than 2-benzylindanocine against colon cancer cells, while 2-benzylindanocine showed more potent activity than indanocine against pancreatic cancer cells. Undoubtedly, both possessed similar anticancer potential. However, reduction of 2-benzylidene to 2-benzyl group did not enhance *in-vivo* efficacy. EAC is a general carcinoma model and not specific to colorectal or pancreatic cancer. However, it is important to mention that amongst the five most potent analogues of indanocine, four were 2-benzylindanocines i.e. **12e**, **12g**, **12i**, and **12m** indicating higher prospects of success while dealing with these compounds.

Benzylindanocine exhibited tubulin polymerisation inhibition. Tubulin-microtubule dynamic equilibrium is an important feature in cell growth and development. Any disturbance in this dynamic equilibrium induces cell cycle arrest [**Negi et al. 2015**]. In molecular docking studies, there were six residual amino acids in binding pocket common to all these ligands. Interestingly, both indanocine and benzylindanocine showed interaction with β:316 and β:318, the crucial residues interacting with target protein. Burns *et al.* (1992) revealed that ALA β:316 is an important residue that is directly involved in the binding of trimethoxyphenyl unit of both

colchicine and podophyllotoxin [**Burns, 1992**]. Although trimethoxyphenyl unit is important for binding to β:316, but hydrophobicity around this residue also plays a role to some extent as it specifies the rate of conformational changes of the ligand.

Deregulatory inflammation plays a pivotal role in the initiation, development and progression of tumour [**Chai et al. 2015**]. The association between chronic inflammation and cancer has been well perceived by epidemiologists since last two decades. both indanocine and benzylindanocine exhibited moderate antiinflammatory activity by down-regulating production of pro-inflammatory cytokines TNF-α and IL-6. Tumour necrosis factor-α (TNF-α) is a key multifunctional cytokine with pleiotropic actions in the regulation of inflammation related immune responses. Being a major cytokine, it regulates other cytokines and chemokines. Hence, TNF-α effectively influences several hallmarks of cancer. Interleukin-6 has gained importance as a prime biomarker in the prognosis of various types of cancer microenvironment. Now it is well established as therapeutic target [**Garbers et al. 2018**]. Antiinflammatory effect of benzylindanocine **12i** is a much desirable attribute to be a broad spectrum anticancer agent.

ADME properties are very much important for a bioactive compound to become a successful drug candidate. ADME properties predicted through SwissADME software were quite satisfactory for both indanocine and 2-benzylindanocine. Water solubility prediction for indanocine was three fold higher than indanocine which indicates that it may have better oral route administration. It did not possess PAINS structure which otherwise might exhibit misleading activity due to artifact [**Baell and Walters 2014**]. Inadequate ADME properties can be devastating to any good drug candidate [**Kenakin TP, 2017**].

Both indanocine and benzylindanocine exhibited significant i*n-vivo* efficacy. However, 2-benzylindanocine displayed moderate activity against EAC carcinoma cells which are considered difficult to break [**Bean & Kassel 1968**]. EAC is a general carcinoma and hence, the evaluation of benzylindanocine against specific rodent models will be done in future.

Benzylindanocine **12i** was safe and well tolerated by Swiss albino mice up to the 1000 mg/kg dose in acute oral toxicity. However, sub-acute and chronic experiments of benzyl indanocine **12i** will be mandatory to be carried out to look for any adverse effect on its prolonged exposure. Safety is an essential component in drug development process and nowadays referred as 'Pharmacovigilance'. It thoroughly focuses on adverse drug reactions. According to WHO, it aims to enhance safety and care of patient in relation to use of medicine and to support public health

programmes by providing reliable, balanced information for effective assessment of risk-benefit profile of medicines [**WHO 2002**].

4. CONCLUSION

In conclusion, an efficient protocol has been developed for the synthesis of indanocine. It is a simple, efficient and straight-forward method. Benzylindanocine **12i** has been investigated as a potent anticancer agent. It exhibited antiproliferative effect *via* microtubule destabilization by occupying colchicine binding pocket of β-tubulin. It showed potential *in-vivo* efficacy and good tolerability in rodent model. The concomitant antiinflammatory activity of benzylindanocine **12i** is paramount, which is very much required in anticancer drug development. Study also enlightens that increase in flexibility at 2-benzylidene system does not necessarily enhance the tubulin binding and hence the efficacy.

5. EXPERIMENTAL

5.1 Chemistry

Synthesis of indanocine

*2,3-Dihydro-5,6-dimethoxy-7-nitro-indan-1-one (**10**)*

To a cold and stirred solution of acetic anhydride (6 mL, 648 mg, 6.4 mmol), nitric acid (3 mL) was added dropwise and reaction mixture was kept in ice bath (3-5 ^{o}C). After 10 min. 5,6-dimethoxyindanone (192 mg, 1 mmol) was added in fractions and further stirred at 8-10 ^{o}C for 2 h. On completion, it was poured into crushed ice, extracted with ethyl acetate (15 mLx3) and washed with water. Combined organic layer was dehydrated over anhydrous Na_2SO_4 and dried *in vacuo*. The desired product was recrystallized with chloroform-hexane to get nitro derivative **10** as amorphous solid.

Yield=74%; light yellowish brown solid, m.p.= 93-95 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.70-2.72 (t, 2H, 3-CH_2, J=6Hz), 3.08-3.10 (t, 2H, 2-CH_2, J=5.75Hz), 3.89 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.02 (s, 1H, 4-CH, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 25.78, 36.69, 56.77, 62.38, 110.09, 120.31, 139.77, 140.69, 152.83, 159.25, 200.34; ESI-MS for $C_{11}H_{11}NO_5$ (MeOH): 238$[M+H]^+$, 260$[M+Na]^+$, 276$[M+K]^+$; ESI-TOF (MeOH) for $C_{11}H_{11}NO_5$ calculated 238.0715 for $[M+H]^+$, found; 238.0718.

*(E)-2-(4-hydroxy-3,5-dimethylbenzyliden)-2,3-dihydro-5,6-dimethoxy-7-nitroindan-1-one (**11i**)*

To a stirred solution of dioxane (20 mL)-conc. HCl (12 N, 2 mL), 5,6-dimethoxy-7-nitroindanone (**10**, 474 mg, 2 mmol) was added. After 10 min., 4-hydroxy-3,5-dimethylbenzaldehyde (328 mg, 2.2 mmol) was added to it and refluxed at 110^{o}C. After 3h, conc. HCl (2 mL) was added to

reaction mixture and further stirred for 5 h. On completion, the reaction mixture was poured into water (20 mL) and extracted with ethyl acetate (20 mLx2). Pooled organic layer was dehydrated over anhydrous Na_2SO_4 and evaporated to dryness. Residue thus obtained was charged on a silica gel glass column and eluted with ethyl acetate-hexane to get the desired benzylidene derivative **11i**. It was recrystallized from chloroform-pentane (1:3).

11i: Yield= 82%; yellow solid; m.p.=220-222 ^{o}C; ^{1}H NMR (500 MHz, $DMSOd_6$): δ 2.22 (s, 6H,CH_3), 3.85 (s, 3H, OCH_3), 4.02 (s,3H, OCH_3), 4.05 (s, 2H, 3-CH_2), 7.31 (s, 2H, 2'& 6'-CH of benzylidene ring), 7.35 (s, 1H, 4-CH, aromatic), 7.56 (s, 1H, 2-benzylidene CH); ^{13}C NMR (125 MHz, $DMSOd_6$): δ 16.64, 16.64, 32.00, 57.21, 62.13, 111.57, 120.19, 124.90, 124.90, 125.66, 130.92, 131.80, 131.80, 134.00, 139.31, 139.45, 148.02, 155.94, 158.41, 187.95; ESI-MS for $C_{20}H_{19}NO_6$ (MeOH): Positive mode: 370 $[M+H]^+$, 408 $[M+K]^+$; Negative mode: 368 $[M-H]^-$; ESI-TOF (MeOH) for $C_{20}H_{19}NO_6$ calculated 370.1290 for $[M+H]^+$, found; 370.1289.

*(E)-2-(4-hydroxy-3,5-dimethylbenzyliden)-2,3-dihydro-5,6-dimethoxy-7-aminoindan-1-one(**1**, Indanocine)*

Nitrobenzylidene derivative **11i** (500 mg, 1.36 mmol) was taken in dry dimethylformamide (10 mL). To this stirred solution 10% palladium-charcoal (50 mg) was added and hydrogen gas was supplied *via* balloon. Reaction mixture was stirred at RT for 30 min. On completion, reaction mixture was carefully filtered through celite (filter aid), washed with dil. HCl (1N, 2 mL) and extracted with ethyl acetate (20 mLx3), washed with water (20 mLx2). Combined organic fraction was dehydrated over anhydrous Na_2SO_4 and dried *in vacuo*. The crude mass was purified through silica gel column eluting with ethyl acetate–hexane to get pure indanocine **1** as amorphous solid.

1: Yield=44%, dark yellowish brown solid; m.p.=236-238 ^{o}C [238-240 ^{o}C]**;** ^{1}H NMR (500 MHz , $DMSOd_6$): δ 2.21 (s, 6H, 2xCH_3), 3.64 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.85 (s,2H,3-CH_2), 6.28 (s, 2H, exchangeable, NH_2), 6.50 (s, 1H, =CH of benzylidene ring), 7.14 (s, 1H, 4-CH of benzylidene ring), 7.28 (s, 2H, 2'& 6'-CH, aromatic), 8.85 (s, 1H, exchangeable, OH, phenolic); ^{13}C NMR (125 MHz, $DMSOd_6$): δ 16.72, 16.72, 31.88, 56.00, 59.45, 97.45, 97.45, 116.33, 124.80, 124.80, 126.42, 130.07, 131.10, 132.14, 133.19, 141.79, 146.88, 154.97, 158.30, 192.96; ESI-MS for $C_{20}H_{21}NO_4$ (MeOH): Positive mode: 340$[M+H]^+$, 362$[M+Na]^+$, 378$[M+K]^+$; Negative mode: 338 $[M-H]^-$; ESI-TOF (MeOH) for $C_{20}H_{21}NO_4$ calculated, 340.1548 for $[M+H]^+$, found, 340.1545.

*General procedure for synthesis of derivatives **11a**, **11c**, **11f-11h**, and **11j-11l***

*(E)-2-benzylidene-2,3-dihydro-5,6-dimethoxy-7-nitroindan-1-one (**11a**)*

To a stirred solution of 3% KOH in methanol (w/w, 10 mL), 5,6-dimethoxy-7-nitroindanone (300 mg, 1.3 mmol) was added. After 10 min, benzaldehyde (170 mg, 1.6 mmol) was added and reaction mixture was further stirred at RT for 4 h. On completion, solvent was evaporated under vacuum and reaction mixture was diluted with water (20 mL). It was extracted with ethyl acetate (20 mLx3), washed with water (20 mLx2) and organic fraction was dehydrated over anhydrous Na_2SO_4. It was dried *in vacuo* to get a crude mass which was purified through column chromatography over silica gel by eluting with ethyl acetate-hexane to get the desired product **11a** as amorphous solid.

11a: Yield=67%; light yellow solid; m.p.=207-209 ^oC; ^1H NMR (500 MHz, DMSOd6):δ 3.86 (s, 3H, OCH_3), 4.04 (s, 3H,OCH_3), 4.12 (s, 2H, 3-CH_2), 7.47-7.52 (bs, 5H, 5xCH of benzylidene ring), 7.75 (bs, 2H, CH of benzylidene & 4-CH); ^{13}C NMR (125 MHz, DMSOd6): δ 31.87, 57.25, 62.13, 111.50, 119.79, 129.05, 129.05, 130.09, 130.80, 130.80, 133.11, 134.51, 134.57, 139.44, 148.34, 158.82, 188.02; ESI-MS for $C_{18}H_{15}NO_5$ (MeOH): Positive mode: 326$[M+H]^+$, 348 $[M+Na]^+$, 364 $[M+K]^+$; Negative mode: 324 $[M-H]^-$; ESI-TOF (CH_3CN) for $C_{18}H_{15}NO_5$ calculated 326.1028 for $[M+H]^+$, found; 326.1027.

*Synthesis of (E)-2-(4-piperazin-1-yl)benzylidene)-2,3-dihydro-5,6-dimethoxy-7-nitro-indan-1-one (**11m**)*

A stirred solution of Boc-piperazine deriv. **11l** (200 mg, 0.39 mmol) in dry dichloromethane (8 mL) was cooled to 5-10 ^oC. To this stirred solution, trifluoroacetic acid (2 mL) was added drop-wise and stirred for an hour. On completion, solvent was evaporated without heating under vacuum. Residue was diluted with water (10 mL), basified with aqueous ammonia (30%, 8-10 mL) and extracted with ethyl acetate (20 mLx2). Pooled organic fraction was washed with water (20 mLx2), dehydrated over anhydrous Na_2SO_4 and dried *in vacuo*. The residue thus obtained was recrystallized from chloroform-acetone to get **11m** as amorphous solid.

11m: Yield=91%; orange solid; m.p.=163-165 ^oC; ^1H NMR (500 MHz, $CDCl_3$+$DMSOd_6$): δ 3.03 (bs, 4H, N-$(CH_2)_2$ of piperazine ring), 3.55 (bs, 4H, N-$(CH_2)_2$ of piperazine ring), 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.06 (s,2H, 3-CH_2), 6.95 (bs, 2H, 2' &3'-CH of 2-benzylidene phenyl ring), 7.45(bs, 2H, 5'& 6'-CH of benzylidene ring), 7.55 (s, 1H, 4-CH, aromatic), 7.68 (s, 1H, 2-benzylidene CH); ^{13}C NMR (125 MHz, $CDCl_3$+$DMSOd_6$): δ 31.37, 44.47, 44.47, 47.12, 47.12, 55.89, 61.25, 109.31, 113.61, 113.61, 120.40, 123.65, 128.87, 131.59, 131.67, 133.50, 133.58, 138.98, 139.17, 146.13, 151.34, 157.53, 187.18; ESI-TOF (MeOH) for $C_{23}H_{23}N_3O_5$ calculated, 410.1715 for $[M+H]^+$, found, 410.1715.

*General procedure for synthesis of 2-benzyl analogues of indanocine **12a-12l***

7-Nitro-2-benzylidene indanocine derivative (**11a-11l**, 1.5 mmol) was taken in dry dimethylformamide (10 mL). To this stirred solution 10% palladium-charcoal (100 mg) was added and hydrogen gas was supplied through a balloon *via* syringe. After 2 h, 50 mg palladium-charcoal was again added to it and reaction was further stirred for 2-4 h at RT. On completion, it was filtered carefully through celite (filter aid), washed with dil. HCl (5%, 5 mL) and extracted with ethyl acetate (20 mLx2). Pooled organic fraction was washed with water, dried over anhydrous Na_2SO_4 and distilled off under vacuum to get a crude mass. It was charged on a silica gel column to get desired 7-amino-2-benzyl indanocine derivative (**12a-12l**) and recrystallized from chloroform-hexane.

*2-(3-Hydroxy-4-methoxybenzyl)-7-amino-5,6-dimethoxy-2,3-dihydroindan-1-one (**12e**)*

Yield=46%, brown solid; m.p.=110-114 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.48-2.70 (bm, 2H, 3-CH_2), 2.81-2.99 (bm, 2H, CH_2 benzylic), 3.22-3.26 (bm, 1H, 2-CH), 3.78 (s,3H, OCH_3), 3.89 (s,3H, OCH_3), 3.91(s, 3H, OCH_3), 5.61 (s, 2H, exchangeable, NH_2), 6.20 (s, 1H, 4-CH, aromatic), 6.69-6.70 (d, 1H, 5'-CH, aromatic, J=8Hz), 6.76-6.78 (d, 1H, 6'-CH, aromatic, J=8Hz), 6.82 (s ,1H, 2'-CH, aromatic); ^{13}C NMR (125MHz, $CDCl_3$): δ 32.10, 36.79, 49.36, 55.92, 55.99, 59.87, 97.59, 110.72, 115.10, 115.38, 120.32, 132.55, 133.27, 141.04, 145.09, 145.50, 151.25, 158.68, 206.98; ESI-MS for $C_{19}H_{21}NO_5$ (MeOH): 344$[M+H]^+$; ESI-TOF (MeOH) for $C_{19}H_{21}NO_5$ calculated, 344.1497 for $[M+H]^+$, found, 344.1493;

*2-(3,4-Methylenedioxybenzyl)-7-amino-5,6-dimethoxy-2,3-dihydroindan-1-one (**12g**)*

Yield=47%, brown solid; m.p.=131-133 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ2.51-2.56 (bm, 2H, phenyl ring CH_2), 2.64-2.68 (bm, 2H, 3-CH_2), 3.21 (m, 1H, 2-CH), 5.61 (s, 2H, exchangeable, NH_2), 5.91 (s, 2H, -*O*-CH_2-*O*-), 6.20 (s,1H, 2'-CH, aromatic), 6.65-6.72 (bs, 3H, 5'& 6'-CH and 4-CH, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.95, 36.50, 49.41, 55.93, 59.87, 97.57, 100.84, 108.18, 109.26, 115.34,121.82, 132.54, 133.70, 141.05, 145.95, 151.16, 158.70, 162.58, 206.79; ESI-MS for $C_{19}H_{19}NO_5$ (MeOH): 364$[M+Na]^+$, 380$[M+K]^+$; ESI-TOF (MeOH) for $C_{19}H_{19}NO_5$ calculated, 342.1341 for $[M+H]^+$, found, 342.1336.

*2-(3,5-Dimethyl-4-hydroxybenzyl)-7-amino-5,6-dimethoxy-2,3-dihydroindan-1-one (**12i**)*

Yield=47.6%, light yellow solid; m.p.=121-123 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ2.22 (s, 6H, 2xCH_3), 2.31-2.40 (bm, 1H, CH_2 benzylic), 2.69-2.70 (bm, 1H, CH_2,benzylic), 2.87-2.99 (bm, 2H, 3-CH_2), 3.20-3.23 (bm, 1H, 2-CH), 3.81 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 5.62 (s, 1H, 2'-CH, aromatic), 6.21 (s, 1H, 6'-CH, aromatic), 6.83 (s, 1H, 4-CH, aromatic); ^{13}C NMR (125MHz,

$CDCl_3$): δ15.98, 15.98, 32.23, 36.71, 49.61, 55.92, 59.87, 97.59, 115.40, 123.04, 123.04, 129.00, 129.00, 131.52, 132.54, 141.04, 150.64, 151.34, 158.67, 207.23; ESI-MS for $C_{20}H_{23}NO_4$ (MeOH): 342$[M+H]^+$, 364 $[M+Na]^+$, 380 $[M+K]^+$; ESI-TOF (MeOH) for $C_{20}H_{23}NO_4$ calculated 342.1705 for $[M+H]^+$, found; 342.1699.

*2-[4-(4-Piperazin-1-yl) benzyl]-7-amino-5,6-dimethoxy-2,3-dihydroindan-1-one (**12m**)*

N-Boc-piperazinyl-2-benzyl indanocine derivative **11m** was used as starting substrate.

Yield=46%, yellow solid; m.p.=137-139 ^oC; ^1H NMR (500MHz, $CDCl_3$): δ2.63-2.67 (bm,2H, CH_2, benzylic), 2.95-3.00 (bm, 2H, 3-CH_2), 3.23-3.26 (t, 1H, 2-CH, J=9Hz), 3.33-3.38 (t, N-$(CH_2)_2$ of piperazine ring, J=12.5Hz), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 5.62 (s,2H, exchangeable, NH_2), 6.20 (s,1H, 4-CH, aromatic), 6.84-86 (d, 2H, 2' & 3'-CH, aromatic, J=8.5Hz), 7.15-7.16 (d, 2H, 5'& 6'-CH, aromatic, J=8.5Hz); ^{13}C NMR (125MHz, $CDCl_3$): δ 32.06, 36.49, 43.49, 43.49, 47.23, 47.23, 49.27, 55.94, 59.89, 97.57, 115.35, 117.47, 117.47, 129.90, 129.90, 132.53, 133.30, 141.04, 148.44, 151.18, 158.71, 206.92; ESI-TOF (MeOH) for $C_{22}H_{27}N_3O_3$ calculated, 382.2130 for $[M+H]^+$, found, 382.2128.

*General procedure for synthesis of 2-benzylidene-7-amino-indanocine analogues (**13n-13p**)*

7-Nitro-2-benzylidene indanocine analogue (**11e**/**11g**/**11p**, 0.6 mmol) was stirred in dry dimethylformamide (10 mL) at RT. To this 10% palladium-charcoal (50 mg) was added and hydrogen was supplied *via* balloon. Reaction mixture was stirred for 4 h. On completion, reaction mixture was carefully filtered through celite, washed with dil. HCl (3 mL), extracted with ethyl acetate and washed with water (20 mLx2). Combined organic layer was dehydrated over anhydrous Na_2SO_4 and evaporated *in-vacuo* to get a residue which was purified through silica gel column eluting with ethyl acetate-hexane to afford desired 7-amino-2-benzylidene indanocine derivatives (**13n**-**13p**).

*(E)-2-(4-Hydroxy-3-methoxybenzylidene)-2,3-dihydro-5,6-dimethoxy-7-amino-indan-1-one (**13n**)*

Yield=46%, brownish yellow solid; m.p.=90-92 ^oC; ^1H NMR (500 MHz, $CDCl_3$): δ 3.81 (s, 3H, OCH_3), 3.85 (s, 2H, 3-CH_2), 3.94 (s, 3H, OCH_3), 3.96 (s,3H, OCH_3), 5.75 (s,2H, exchangeable, NH_2), 6.38 (bs, 1H, 2'- CH, aromatic), 6.97-6.99 (d, 1H, 5'-CH, aromatic, J=8Hz), 7.11-7.11 (d, 1H, 6'-CH, aromatic, J=1.5Hz), 7.26 (s, 1H, 4-CH, aromatic), 7.40 (s, 1H, =CH, 2-benzyidene); ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.35, 55.98, 56.01, 59.94, 97.37, 112.96, 114.89, 117.42, 124.31, 128.38, 130.85, 132.84, 133.88, 141.66, 146.65, 146.76, 146.90, 158.42, 193.85; ESI-MS for $C_{19}H_{19}NO_5$ (MeOH): Positive mode: 342$[M+H]^+$, 364$[M+Na]^+$, 380$[M+K]^+$; Negative mode: 340$[M-H]^-$; ESI-TOF (MeOH) for $C_{19}H_{19}NO_5$ calculated 342.1341 for $[M+H]^+$, found, 342.1334.

*Synthesis of 5,6-dimethoxy-1-oxo-indan-2-yl-2-acinitro-methanium (**14**)*

To a cold stirred solution of sodium nitrite (50 mg) in dry methanol (6 mL), boron trifluoride-etherate (0.5 mL) was added dropwise. To this reaction mixture, a solution 5,6-dimethoxyindanone (**9**, 50 mg, 0.26 mmol) pre-dissolved in methanol (1 mL) was added dropwise to it. The reaction mixture was further stirred on ice-bath for 2 h followed by at RT for an hour. On completion, solvent was evaporated under vacuum and residue was taken in ethyl acetate (20 mL), organic layer was washed with water and dehydrated over anhydrous Na_2SO_4. It was evaporated under vacuum to get a residue which was recrystallized from acetone-chloroform to get compound **14** as amorphous solid.

14: Yield=61%, yellowish brown solid; m.p.=204-206 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$+DMSOd6): δ3.89 (s, 3H, OCH_3), 4.38 (s, 3H, OCH_3), 4.48 (s, 2H, 3-CH_2), 7.48 (s, 1H,4-CH, aromatic), 7.71 (s, 1H, 7-CH), 8.21 (s,1H, OH); ^{13}C NMR (125MHz, $CDCl_3$+DMSOd6): δ 26.86, 49.32, 54.93, 55.26, 103.64, 106.89, 130.03, 141.42, 148.45, 153.56, 155.11, 187.23; ESI-TOF (MeOH) for $C_{11}H_{11}NO_5$, calculated, 238.0715 for $[M+H]^+$, found, 238.0852.

5.2. Biological evaluations

Detailed protocols are in Supplementary information.

Antiproliferative activity by Sulphorhodamine assay

Antiproliferative assay was performed as per reported method [**Akindele et al. 2015**]. Combretastatin A4 and paclitaxel were used as standard drugs for comparison.

Tubulin polymerisation inhibition assay

Tubulin polymerization assay was performed using 'assay kit-BK-006P' from Cytoskeleton, USA, as per Manufacturer's protocol [**Lee et al. 1997**]. Paclitaxel was used as a standard stabilizer of tubulin polymerase and podophyllotoxin (PDT) as standard inhibitor.

Anti-inflammatory activity against LPS-induced inflammation

Antiinflammatory assay was performed on primary macrophages cells which were isolated from peritoneal cavities of Swiss albino mice as per our reported method [**Pathak et al. 2019**]. TNF-α and IL-6 released in the cell culture supernatant were determined with ELISA kits.

Molecular docking studies

Docking studies were performed to know the binding conformation of ligands in active site of protein by AutoDockVina 1.1.2 version by keeping all the docking parameters on default settings [**Trott and Olson 2010**]. The bound protein 3D crystallographic structure of Tubulin-colchicine complex PDB ID:4O2B was used to know the binding of enantiomers (2R/S)

benzylindanocine**12i**, indanocine along with colchicine and podophyllotoxin as control drugs. Whole protein was selected with maximum grid size for docking. All ligands and protein structures were prepared for docking studies by protonating them and removing water molecules from protein. After docking top 10 ligand protein interaction poses were selected and visualize for their 2D and 3D poses using Discovery Studio visualizer. Further, ADME properties were predicted at free online software provided by Swiss Institute of Bioinformatics, Switzerland dated 26/04/2020 [**www.swissadme.ch 2020**].

Ehrlich Ascites carcinoma

In vivo anticancer activity of benzylindanocine (**12i**) was evaluated against EAC mice model. Study and number of animals used were approved by Institutional Animal Ethics Committee of CSIR-CIMAP, Lucknow, India *via* CIMAP/IAEC/2016-19/32 dated 09-02-2017.

Acute oral toxicity in Swiss albino mice

Safety evaluation of compound **12i** was done as per reported method [**Chanda et al. 2009**] at four different single acute oral doses at 5 mg/kg, 50 mg/kg, 300 mg/kg, and 1000 mg/kg. Study and number of animals used were approved *via* CIMAP/IAEC/2016-19/01 dated 09-02-2017 by Institutional Animal Ethics Committee of CSIR-CIMAP, Lucknow, India.

4.3.7. Statistical analysis

All data have been expressed as mean±SD and calculated using MS-Excel. Statistical analysis of differences was carried out by ANOVA followed by Tukey's multiple comparison test. Comparisons were made relative to the untreated controls. Differences with p value<0.05 were considered significant.

Declaration of competing interest

Authors declare that there is no conflict of interest.

Acknowledgement

The study was financially supported from CSIR CIMAP under MLP-10. Ms Sadiya Khwaja and Ms. Kaneez Fatima acknowledge to CSIR for Senior Research Fellowships. Biological Central Facility and Chemical Central Facility, CSIR-CIMAP are duly acknowledged for Sophisticated Instruments support.

Supplementary information

Additional information may be found online in the Supporting Information section

REFERENCES

Akindele, A. J., Wani, Z., Mahajan, G., Sharma, S., Aigbe, F. R., Satti, N., Adeyemi, O. O., & Mondhe, D. M. (2015). Anticancer activity of *Aristolochia ringens* Vahl. (Aristolochiaceae). *J. Trad. Compl. Med.*, *5*, 35-41.

Baell, J., & Walters, M. A. (2014). Chemistry: Chemical con artists foil drug discovery. *Nature*, *513*, 481-483.

Beams, H. W., & Kessel, R. G. (1968). Properties of the Ehrlich Ascites tumor cell as determined by electron microscopy, ultracentrifugation, and hydrostatic pressure. *Cancer Res. 28* , 1944–1951.

Burns R. G. (1992). Analysis of the colchicine-binding site of beta-tubulin. *FEBS J*, *297*, 205-208.

Cancer facts and figures (2020), American Cancer Society, Atlanta, GA 30303-1002, USA.

Cancer Keyfacts (2018). WHO, 12 September 2018.

Carson, D. A., Shih, C. H., Cottam, H. B., & Leoni, L. (2000). Indanone tetralone compounds for inhibiting cell proliferation. US Pat., *6*,162,810.

Chai, E. Z. P., Siveen, K. S., Shanmugam, M. K., Arfuso, F., & Sethi, G. (2015). Analysis of the intricate relationship between chronic inflammation and cancer. *Biochem. J., 468,* 1–15.

Chanda, D., Shanker, K., Pal, A., Luqman, S., Bawankule, D. U., Mani, D. N., & Darokar, M. P.(2009). Safety evaluation of Trikatu, a generic Ayurvedic medicine in Charles Foster rats. *J. Toxicol. Sci.*, *34*, 99-108.

Coulup, S. K., & George, G. I. (2019). Revisiting microtubule targeting agents: α-tubulin and the pironetin binding site as unexplored targets for cancer therapeutics. *Bioorg. Med. Chem. Lett.*, *29*, 1865-1873.

Das, L., Gupta, S., Dasgupta, D., Poddar, A., Janik, M. E., & Bhattacharyya, B. (2009). Binding of indanocine to the colchicine site on tubulin promotes fluorescence, and its binding parameters resemble those of the colchicine analogue AC. *Biochemistry*, *48*, 1628–1635.

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., franceschi, C., Ferrucci, L., Gilroy, D.W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nat. Med.*, *25*, 1822-1832.

Garbers, C., Heink, S., Korn, T., & Rose-John, S. (2018). Interleukin-6: designing specific therapeutics for the complex cytokine. *Nat. Rev. Drug Discov.*, *17*, 395-412.

Giannakakou, P., Sackett, D., & Fijo, T. (2000). Tubulin/microtubule: still a promising target for new chemotherapeutic agents. *J. Natl. Cancer Inst.*, *92*, 182-183.

http://www.swissadme.ch/, Swiss Institute of Bioinformatics 2019, access date 26/04/2020.

Huang, L., Lu, C., Sun, Y., Mao, F., Luo, Z., Su, T., Jiang, H., Shan, W., & Li, X. (2012). Multitarget-directed benzylideneindanone derivatives: anti-β-amyloid (Aβ) aggregation, antioxidant, metal chelation, and monoamine oxidase B (MAO-B) inhibition properties against Alzheimer's disease. *J. Med. Chem.*, *55*, 8483–8492.

Kapoor, S., & Panda, D. **(**2012). Kinetic stabilization of microtubule dynamics by indanocine perturbs EB1 localization, induces defects in cell polarity and inhibits migration of MDA-MB-231 cells. *Biochem. Pharmacol.*, *83*, 1495–1506.

Kaul, R., Risinger, A. L., & Mooberry, S. L. (2019). Microtubule-targeting drugs: More than antimitotics. *J. Nat. Prod.* , *82*, 680-685.

Kenakin T. P. (2017). In Book: Pharmacology in drug discovery and development. Chapter 7: Pharmacokinetics I: Permeation and metabolism", Second edition, Academic Press, Elsevier Inc., pp.157-191.

Lee, J. C., & Timassheff, S. N. (1997). *In vitro* reconstitution of calf brain microtubules: effects of solution variables. *Biochemistry*, *16*, 1754-1764.

Leoni, L. M., Hamel, E., Genini, D., Shih, H., Carrera, C. J., Cottam, H. B., & Carson, D. A. (2000). Indanocine, a microtubule-binding indanone and a selective inducer of apoptosis in multidrug-resistant cancer cells. *J. Natl. Cancer Inst.*, *92*, 217–224.

Negi, A. S., Gautam, Y., Alam, S., Chanda , D., Luqman, S., Sarkar, J., Khan, F., & Konwar, R. (2015). Natural antitubulin agents: importance of 3,4,5-trimethoxyphenyl fragment. *Bioorg. Med. Chem.*, *23*, 373-389.

Pathak, N., Fatima, K., Singh, S., Mishra, D., Gupta, A. C., Kumar, Y., Chanda, D., Bawankule, D. U.,

Shanker, K., Khan, F., Gupta, A., Luqman, S., & Negi, A. S. (2019). Bivalent furostene carbamates as antiproliferative and antiinflammatory agents. *J. Steroid Biochem. Mol. Biol.*, *194* Article no. 105457.

Shih, H. C., Deng, L., Carrera, C. J., Adachi, S., Cottam, H. B., & Carson, D. A. (2000). Rational design, synthesis and structure-activity relationships of antitumore (E)-2-benzylidene-1-tetralones and (E)-2-benzylidene-1-indanones. *Bioorg. Med. Chem. Lett.*, *10*, 487–490.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.*, *31*, 455-461.

Tunbridge, G. A., Oram, J., & Caggiano, L. (2013). Design, synthesis and antiproliferative activity of indole analogues of indanocine. *Med. Chem. Commun.*, *4*, 1452-1456.

WHO (2002). The Importance of Pharmacovigilance, safety monitoring of medicinal products. World Health Organization.

…….

Table 1A: Antiproliferative activity of indanocine derivatives (% Inhibition).

Sample code	Conc. (μM)	Cytotoxicity (% inhibition)[a,*]			
		HCT-116	MIA PACA-2	SW 620	A549
11b	10	---	3.14± 0.077	14.94± 0.027	---
	50	---	55.95± 0.043	51.39± 0.031	---
11e	10	23.27 ± 0.064	---	42.14± 0.331	---
	50	50.81 ±0.102	36.01± 0.093	51.32± 0.048	20.57± 0.085
11f	10	16.42 ±0.157	31.63± 0.019	41.85± 0.052	20.26± 0.152
	50	49.43 ± 0.075	41.54± 0.034	53.98± 0.036	26.1± 0.130
11k	10	11.8±0.056	27.47± 0.024	19.67± 0 .021	24.58± 0.088
	50	56.8±0.060	42.86± 0.035	30.61± 0.059	31.78± 0.067
12a	10	9.94 ± 0.160	17.71± 0.127	22.29± 0.049	5.53± 0.042
	50	36.97± 0.102	50.19± 0.050	33.88± 0.110	9.68± 0.036
12d	10	49.35± 0.051	30.83± 0.070	17.65± 0.169	19.9± 0.036
	50	57.24± 0.008	48.31± 0.049	39.15± 0.053	20.79± 0.028
12e	10	**51.84± 0.015**	42.26± 0.024	27.6± 0.212	23.01± 0.036
	50	56.52± 0.050	60.38± 0.013	45.41± 0.067	28.24± 0.023
12f	10	30.46± 0.027	42.95± 0.059	42.06± 0.056	16.26± 0.125
	50	61.26± 0.076	52.33± 0.037	54.3± 0.070	21.82± 0.174
12g	10	**50.42± 0.042**	39.49± 0.049	20.03± 0.163	14.01± 0.023
	50	53.81± 0.035	53.41± 0.033	41.13± 0.176	22.94± 0.033
12h	10	35.51± 0.129	40.25± 0.092	16± 0.033	21.57± 0.014
	50	55.65± 0.087	55.63± 0.031	48.02± 0.006	30.01± 0.044
12i (Benzylindanocine)	**10**	**57.06± 0.056**	**63.48± 0.014**	34.89± 0.140	6.5± 0.122
	50	61.91± 0.017	66.94± 0.015	44.8± 0.183	9.99± 0.157
12l	10	24.25± 0.029	44.04± 0.128	34.31± 0.041	18.26± 0.016
	50	26.11± 0.085	64.89± 0.005	47.47± 0.058	37.93± 0.038
12m	10	**55.51± 0.029**	43.15± 0.094	31.36± 0.053	19.09± 0.012
	50	67.99± 0.003	65.15± 0.046	37.86± 0.091	50.83± 0.019
13o	10	22.4±0.072	28.69± 0.069	36.99± 0.087	19.2± 0.071
	50	38.6±0.077	50.72± 0.115	42.8± 0.152	19.23± 0.070
13p (1:Indanocine)	**10**	**66.13±0.007**	**59.59± 0.069**	28.82± 0.042	7.02± 0.024
	50	68.13 ± 0.013	62.51± 0.047	47.85± 0.007	12.84± 0.023
CA4	**10**	**61.93± 0.012**	**63.31± 0.019**	39.45± 0.027	30.65± 0.016
	50	67.51± 0.013	68.8± 0.011	40.91± 0.060	35.85± 0.025
Paclitaxel	10	48.87± 0.045	28.99± 0.039	28.35± 0.119	27.45± 0.056

	50	53.64± 0.023	29.37± 0.043	51.32± 0.044	36.39± 0.059

* %inhibition<50% at 50 µM was considered inactive, *p<0.05 as compared to control

Table 1B: Cytotoxicity (IC_{50}) of indanocine (**1**) and benzylindanocine (**12i**)

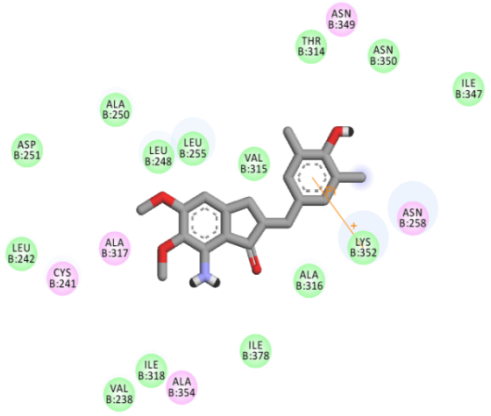
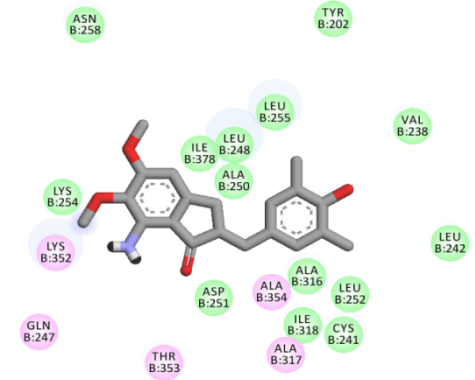
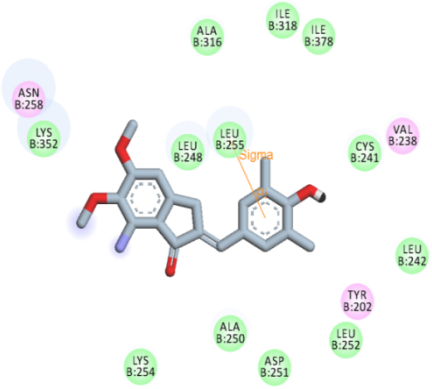
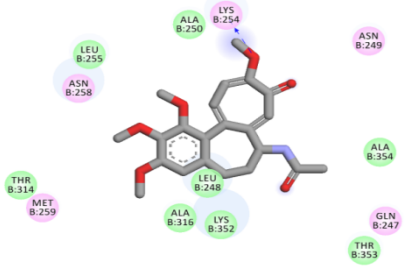
Compound	**Cytotoxicity IC_{50} in µM**	
	HCT- 116	**MIA PACA-2**
Indanocine (**1**)	1.032 ±0.142	0.438 ±0.190
Benzylindanocine (**12i**)	1.295 ±0.232	0.577 ±0.208
Combretastatin A4	<0.1	<0.1

Table 2. Effect of indanocine (**1**) and benzylindanocine (**12i**) on the pro-inflammatory cytokine TNF-α and IL-6 levels (mean±SEM: *P<0.05; Vehicle vs treatment; #Vehicle vs normal; n=3)

Compound	**LPS (0.1µg/mL)**	**Dose (µg/mL)**	**TNFα (pg/mL)**	**TNF-α % inhibition**	**IL-6 (pg/mL)**	**IL-6 % inhibition**
Normal	-	-	348.89±92.74	NA	1222±201.34	NA
Vehicle	✓	-	1049.62±19.59#	---	8505±400.02#	---
Benzylindanocine (**12i**)	✓	1	993.41±78.08	5.35±7.43	5285±506.44*	37.85±5.95
	✓	10	751.73±76.98*	**28.38±7.33**	4591±526.04*	**46.02±6.18**
Indanocine (**1**)	✓	1	839.51±28.62	20.01±2.72	5360±355.16*	36.97±4.17
	✓	10	741.36±30.55*	**29.36±2.91**	4982±418.39*	**41.41±4.91**
Dexamethasone	✓	1	654.2±42.82*	**37.67±4.08**	2582±114.29*	**69.63±1.34**

Table 3: Interaction of indanocine and both enantiomers of benzylindanocine with β-tubulin (PDB ID:402B)

S. No.	**Compounds**	**Docking Energy Kcal/Mol**	**Interacting amino acids**	**Docking 2D poses**

1	(R)-Benzylindanocine (**12i**-2α-isomer)	-7.9	VAL B:238, CYS B:241, LEU B:242, LEU B:248, ALA B:250, ASP B:251, LEU B:255, ASN B:258, THR B:314, VAL B:315, ALA B:316, ALA B:317, ILE B:318, ILE B:347, ASN B:349, ASN B:350, LYS B:352, ALA B:354, ILE B:378	
2	(S)-Benzylindanocine (**12i**-2β-isomer)	-8.1	TYR B:202, VAL B:238, CYS B:241, LEU B:242, GLN B:247, LEU B:248, ALA B:250, ASP B:251, LEU B:252, LYS B:254, LEU B:255, ASN B:258, ALA B:316, ALA B:317, ILE B:318, LYS B:352, THR B:353, ALA B:354, ILE B:378	
3	Indanocine (**1**)	-8	TYR B:202, VAL B:238, CYS B:241, LEU B:242, LEU B:248, ALA B:250, ASP B:251, LEU B:252, LYS B:254, LEU B:255, ASN B:258, ALA B:316, ILE B:318, LYS B:352, ILE B: 378	
4	Colchicine	-6.2	GLN B:247, LEU B:248, ASN B:249, ALA B:250, LEU B:255, LYS B:254, ASN B:258, MET B:259, THR B:314, ALA B:316, LYS B:352, THR B:353, ALA B:354	

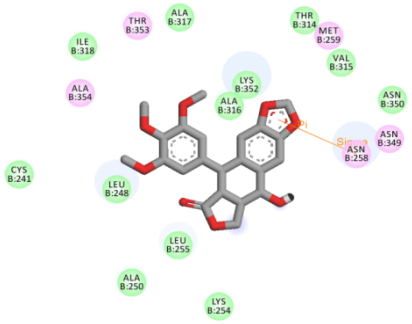
5	Podophyllotoxin	-7	CYS B:241, LEU B:248, ALA B:250, LYS B:254, LEU B:255, MET B:259, ASN B:258, THR B:314, VAL B:315, ALA B:316, ALA B:317, ILE B:318, ASN B:349, ASN B:350, LYS B:352, THR B:353, ALA B:354	

Table 4 : Various druggability parameters of indanocine (**1**) and 2-benzylindanocine (**12i**)

Parameter	**1**	**12i**	**Acceptable range**	**Parameter**	**1**	**12i**	**Acceptable range**
Physicochemical properties				**Lipophilicity**			
Molecular formulae	C20H21NO4	C20H23NO4	---	Log $P_{o/w}$	3.29	3.23	≤5
M.Wt.	339.39	341.40	≤500	**Pharmacokinetics**			
Retable bonds	3	4	≤10	GI absorption	High	High	---
H-bond acceptors	4	4	≤10	BBBpermeability	No	No	---
H-bond donors	2	2	≤5	P-gp substrate	No	No	---
Molar Refractivity	98.44	98.13	40-130	CYP1A2 inhibition	Yes	Yes	---
Sp3 hybridisation fraction	0.25	0.35	Not less than 0.25	CYP2C9/19 inhibition	Yes	Yes	---
TPSA	81.78Å^2	81.78Å^2	20 Å^2 to 130 Å^2	**Drug likeness**			
Water solubility				Lipinski rule	Yes, no violation	Yes, no violation	Up to 1 violation
Water solubility	3.85 µg/mL	11.6 µg/mL		Bioavailability score	0.55	0.55	moderate
Solubility class	Moderate	Moderate	acceptable	**Medicinal chemistry**			
Log S	-4.61	-4.47	>-4	PAINS	No	No	No

Table 5a. Effect of indanocine (**1**) and 2-benzylindanocine (**12i**) on body weight of mice bearing EAC

Treatment	Dose	Body weight (g)			
		Day 1	Day 5	Day 9	Day 12
Control	Normal saline (0.2 mL i.p.)	26.60±0.75	27.28±0.86	33.32±1.31	34.30±1.28
Benzylindanocine (**12i**)	10 mg/kg i.p.	25.4±1.77	25.24±1.89	27.964±1.63	28.6±1.76
Benzylindanocine (**12i**)	30 mg/kg i.p	23.75±1.77	25.875±0.62	22.655±1.97	24.4±2.23
Benzylindanocine (**12i**)	90 mg/ kg i.p.	25.4±1.77	26.66±2.07	31.476±1.65	32.56±1.66
Indanocine (**1**)	30 mg/kg i.p.	25±1.22	25.88±1.14	28.386±1.19	28.32±1.22
5-Fluorouracil	20 mg/kg i.p.	27.6±0.24	27.24±0.49	28.582±0.45	29±0.45

Table 5b. *In vivo* efficacy of indanocine (**1**) and 2-benzylindanocine (**12i**) against EAC

Treatment	Dose	Day 12				
		Av. Volume of ascitic fluid (mL)	Av. Weight of ascitic fluid (g)	Av. No. of tumor cells (1×10^7)	% Tumor growth inhibition	Mortality
Control	Normal saline (0.2 mL i.p.)	4.28±0.37	3.94±0.37	92.60±20.63	---	**0**
Benzylindanocine (**12i**)	10 mg/kg i.p.	3.52±0.13	3.28±0.09	55.4±2.06	**40.2**	**0**
Benzylindanocine (**12i**)	30 mg/kg i.p.	3.075±0.13	3.175±0.07	42.75±5.15	**53.8**	**0**
Benzylindanocine (**12i**)	90 mg/kg i.p.	2.54±0.43	2.42±0.49	20±1.48	**78.4**	**0**
Indanocine (**1**)	30 mg/kg i.p.	0.76±0.05	0.532±0.07	10.1±1.27	**89.1**	**0**
5-Fluorouracil	20 mg/kg i.p.	0.58±0.09	0.2±0.04	2.55±0.42	**97.2**	**0**

***p<0.001 (Dunnett test);

Parameters		Dose of 2-benzylindanocine (12i) at mg/kg body weight as a single oral dose				
		Control	**5 mg/kg**	**50 mg/kg**	**300 mg/kg**	**1000 mg/kg**
Body weight (gm)		24.19±1.19	21.09±0.28	22.30±0.58	21.30±0.65	21.29±0.63
Haematological profile	**Haemoglobin (gm/dL)**	13.81±0.95	13.27±0.27	15.30±0.59	14.16±0.64	14.56±0.82
	RBC (million/mm^3)	6.48±0.74	5.97±0.29	6.06±0.53	7.05±0.59	5.70±0.67
	WBC (1000*/mm^3)	6.52±0.83	4.80±0.46	4.24±0.67	3.91±0.34*	3.33±0.32*
Liver Function Test	**ALKP (U/L)**	80.35±6.36	75.97±2.46	77.56±8.42	60.05±4.19	73.11±3.82
	SGOT (U/L)	33.52±2.61	30.69±3.06	27.32±1.45	25.27±2.17	25.54±2.01
	SGPT (U/L)	15.92±0.90	14.21±0.71	14.72±0.39	13.98±0.56	11.60±0.93
	Albumin (g/dL)	2.70±0.13	2.67±0.06	2.76±0.03	2.55±0.05	2.62±0.05
	Bilirubin (mg/dL)	0.47±0.04	0.48±0.03	0.58±0.04	0.46±0.05	0.56±0.05
	Serum Protein (mg/ml)	3.48±0.06	3.42±0.04	3.43±0.05	3.14±0.24	3.39±0.03
Kidney Function Test	**Creatinine (mg/dL)**	0.37±0.02	0.35±0.03	0.32±0.02	0.36±0.04	0.30±0.03
Lipid Profile	**Triglycerides (mg/dL)**	87.52±6.76	74.18±4.64	85.21±3.50	81.85±5.33	93.94±7.80

Table 6. Effect of 2-benzylindanocine (**12i**) as a single acute oral dose in Swiss albino mice (Mean±SE; n=6; *, significant compared to control P<0.05).

Figure legends:

Figure 1. Structures of some of the important antimitotic anticancer drugs

Figure 2: Structure and antiproliferative activity of indanocine and some previously reported derivatives

Figure 3A: Effect of indanocine (**1**) (5, 10 and 20 µM) on tubulin polymerisation

Figure 3B: Effect of 2-benzylindanocine (**12i**) (5, 10 and 20 µM) on tubulin polymerisation

Figure 4. Molecular docking representation of compounds binding within the same binding pocket. The compounds were represented in color as 2α-benzylindanone (**12i)-** (cyn), 2β-benzylindanone (**12i)** (yellow), indanocine (green), colchicine (red), and podophyllotoxin (blue)

Figure 5: Two dimensional bioavailability radars of Indanocine (**1**) and 2-benzylindanocine (**12i**)

(Six physicochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation. The pink area represents the optimal range for each properties (lipophilicity: XLOGP3 between − 0.7 and + 5.0, size:MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 A^2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds. In this example, both the compounds are predicted to be moderate orally bioavailable)

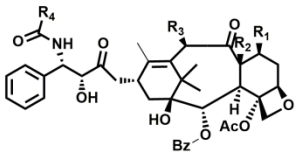
Figures 6 A & 6B: Effect of benzylindanocine **12i** on absolute and relative organ weight in Swiss albino mice (Mean±SE; n=6).

Figure 7: Effect of benzylindanocine **12i** on differential leucocytes counts in Swiss albino mice (Mean±SE; n=6).

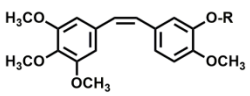
Figure 7B. Effect of **22** as a single acute oral dose at 5, 50, 300 and 1000 mg/kg body weight on differential leucocytes counts in Swiss albino mice (Mean±SE; n=6).

Scheme 2:

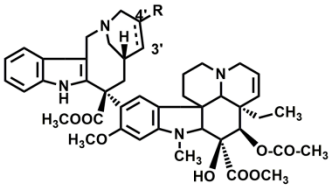
Reagents and conditions: iv) Ac_2O-HNO_3, 5-10^oC, 3h, 74%; v) $NaNO_2$-dry MeOH, BF_3-Et_2O, 10^oC-RT, 2h, 61%; vi) For **11a**, **11c**, **11f-11h** &**11j-11l**: substituted benzaldehyde, 3% KOH in MeOH, 3-6h, 59-86% **11a**,67%; **11c**,59%; **11f**, 86%; **11g**, 61%; **11h**,65%;&**11j**, 56%, **11k**, 69%; **11l**, 76%; For **11b**, **11d**, **11e**, &**11i**: substituted benzaldehyde, dioxane-HCl, 110^oC, 7-8h, **11b**, 69%; **11d**, 83%; &**11e**, 64%;**11i**, 82%. EtOH-$SOCl_2$, RT, 20h, %; For **11m**: **11l**, dry DCM-TFA, 0^oC,1h, 91%; vii) 10%Pd-C, H_2, RT, 2-4h, **12a-12m**: 44-67%; **13n-13p**: 44-46%.



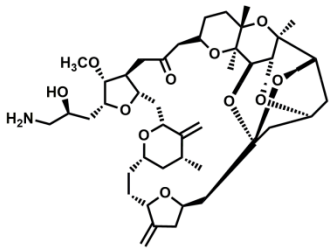
	R_1	R_2	R_3	R_4
Paclitaxel	OH	CH_3	Ac	Ph
Docetaxel	OH	CH_3	OH	-O-t-But
Cabazitaxel	OCH_3	CH_3	OCH_3	-O-t-But
Larotaxel	-----CH_2-----		OAc	-O-t-But



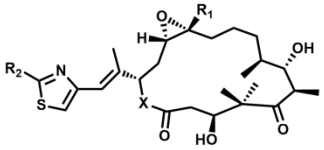
Combretastatin A4: R=H;
Combretastatin A4P: R=P(O)$(ONa)_2$



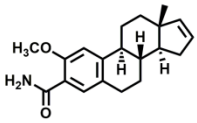
Vinblastine, R=4'-hydroxy, 4'ethyl;
Vinoflunine, R= 1,1-difluoroethyl;
Vinorelbine, R=Ethyl & 3'-4'-ene



Eribulin mesylate

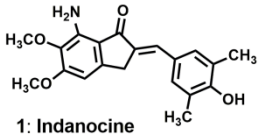


Ixabepilone: X=NH, R_1=CH_3, R_2=CH_3;
Epothilone B: X=O, R_1=CH_3, R_2=CH_3
BMS-310705: X=O, R_1=CH_3, R_2=NH_2



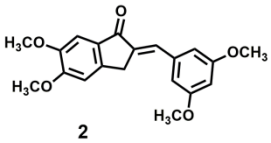
ENMD-1198

cbdd_13857_f1.tif



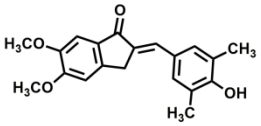
1: **Indanocine**

MCF-7, IC_{50}=0.004 μM; **MDA-MB-231**, IC_{50}=0.009 μM;
HL-60, IC_{50}=0.002 μM; **KB-3-1,** IC_{50}=0.007 μM;
Jurkat, IC_{50}=0.001 μM; **K562**, IC_{50}=0.010 μM;
HCT116, IC_{50}=0.010 μM; **Antitubulin**, IC_{50}=1.7 μM;



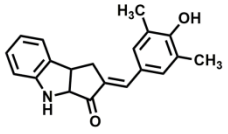
2

Jurkat, IC_{50}=0.008 μM;



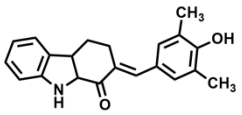
3: Indanorine

Jurkat, IC_{50}=0.006 μM;



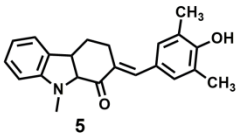
3: Indolocine

MCF-7, IC_{50}=0.7 μM;
MDA-MB-435, IC_{50}=0.16 μM;
OVCAR-3, IC_{50}=0.51 μM;
K562, IC_{50}=0.33μM



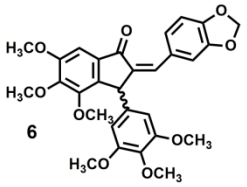
4

MCF-7, IC_{50}=0.35 μM;
MDA-MB-435, IC_{50}=0.12 μM;
OVCAR-3, IC_{50}=0.33 μM;
K562, IC_{50}=0.32 μM;
T47D, IC_{50}=0.74 μM



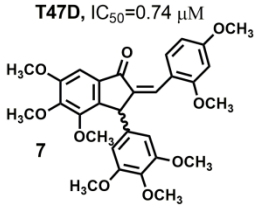
5

MCF-7, IC_{50}=0.37 μM;
MDA-MB-435, IC_{50}=0.16 μM;
OVCAR-3, IC_{50}=0.35 μM;
K562, IC_{50}=0.33 μM;



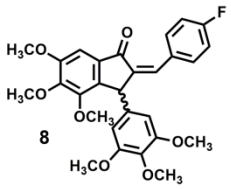
6

MCF-7, IC_{50}=0.01 μM



7

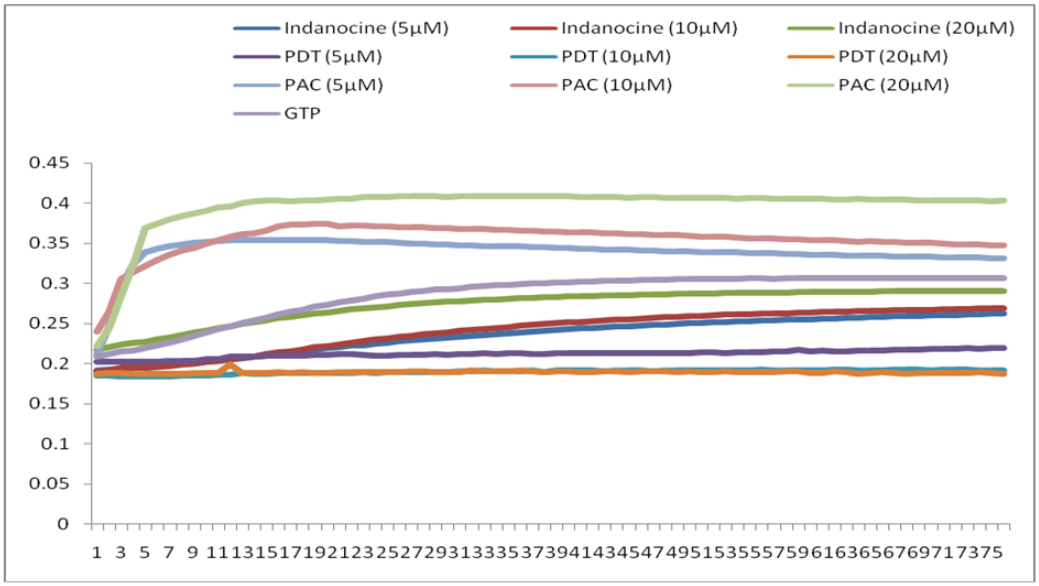
MCF-7, IC_{50}=1 μM



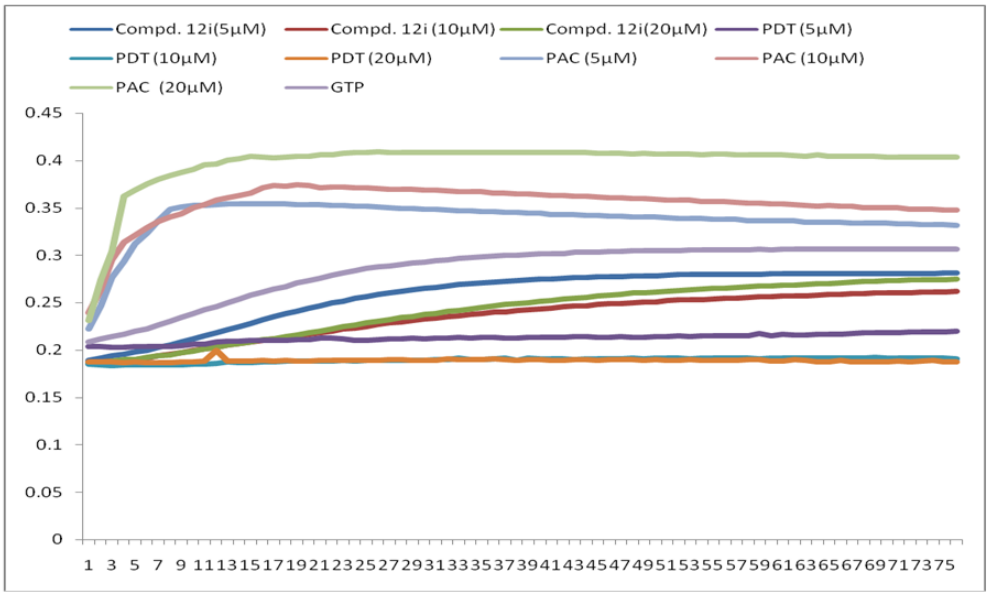
8

MCF-7, IC_{50}=0.68 μM

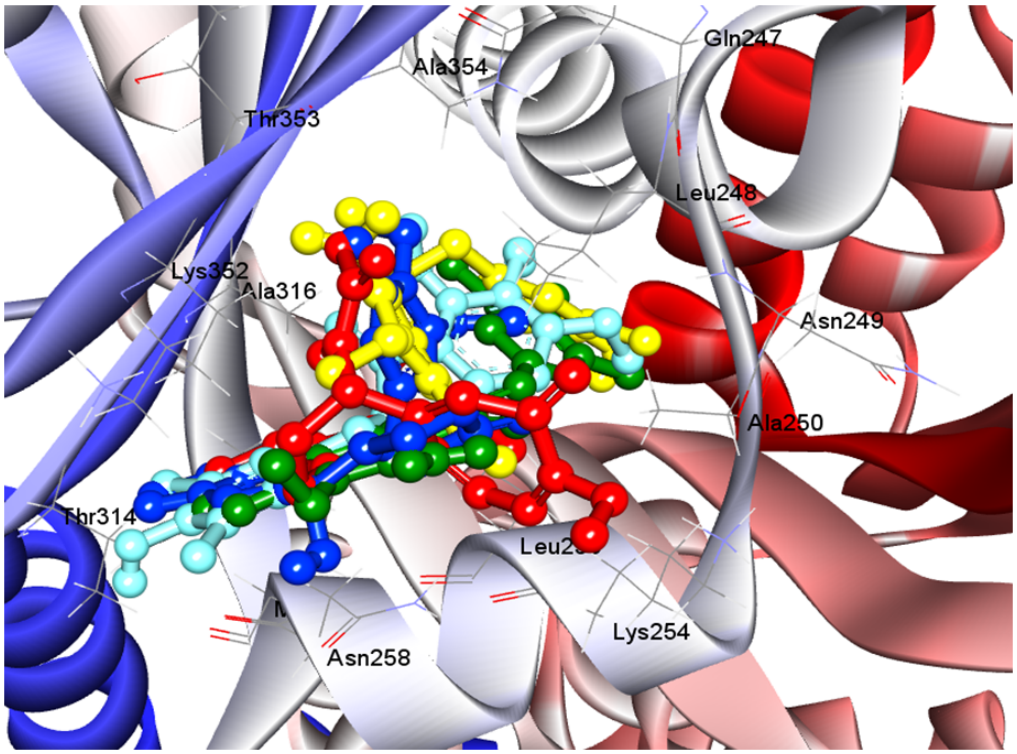
cbdd_13857_f2.tif



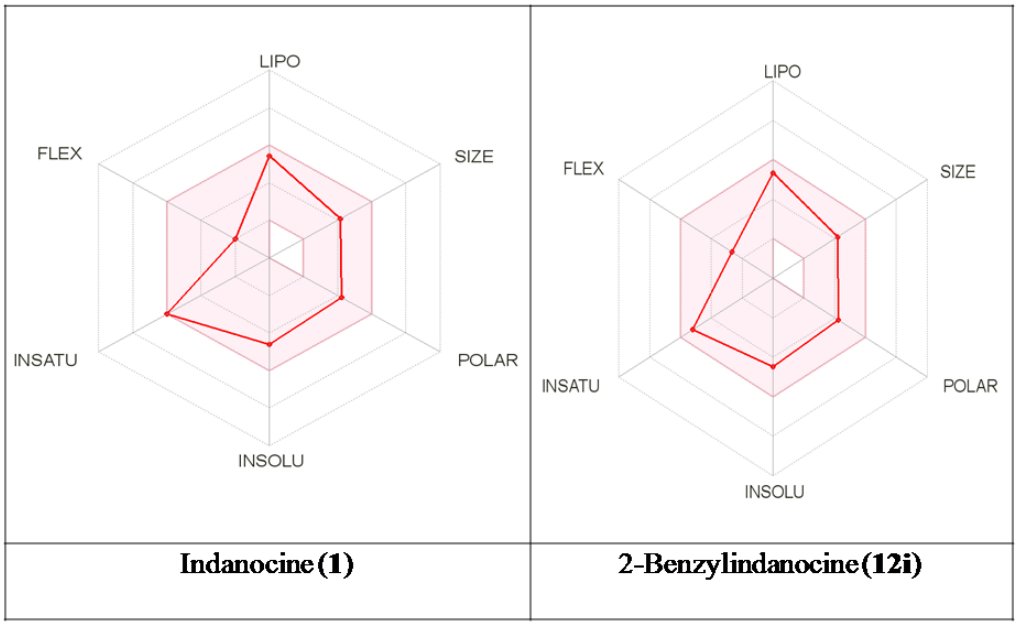
cbdd_13857_f3a.tif



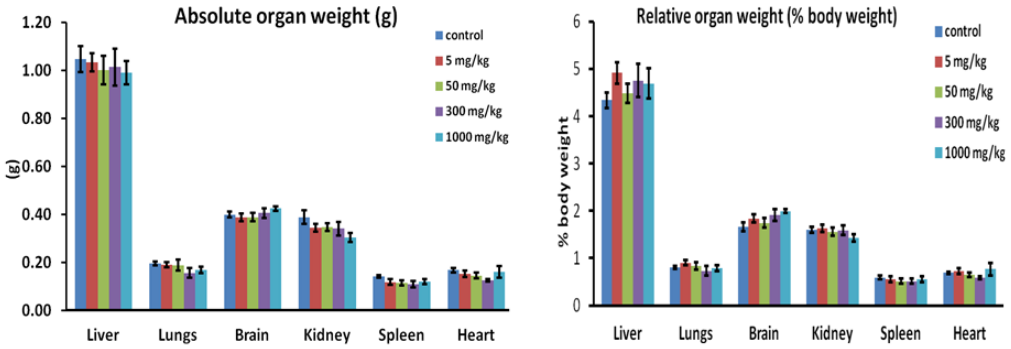
cbdd_13857_f3b.tif



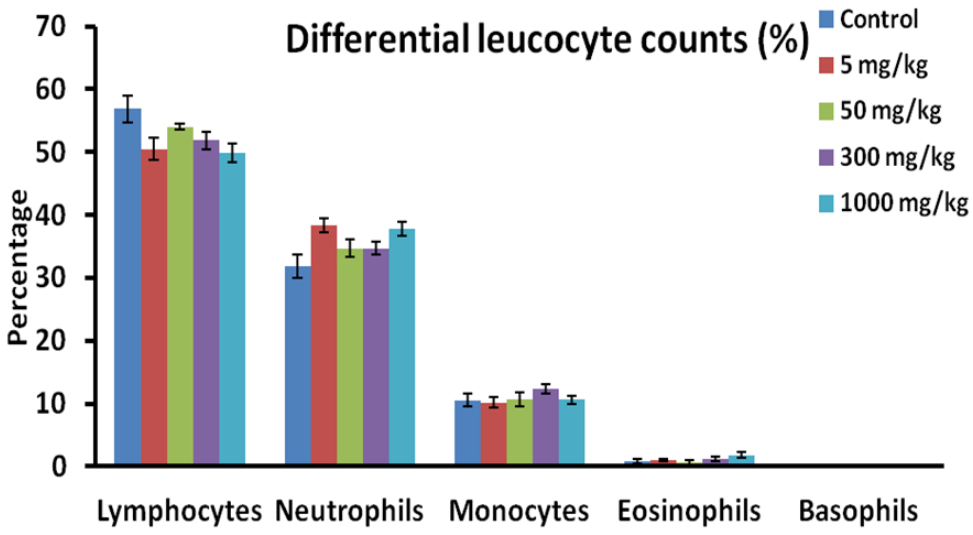
cbdd_13857_f4.tif



cbdd_13857_f5.tif

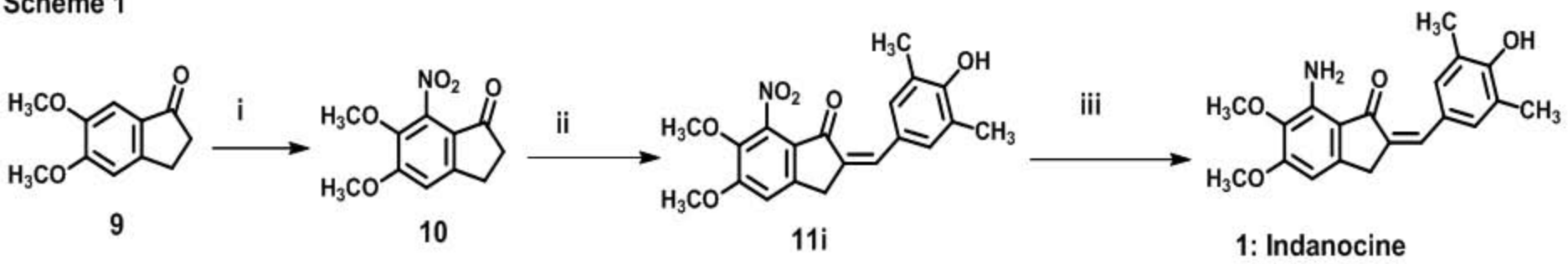


cbdd_13857_f6.tif



cbdd_13857_f7.tif

Scheme 1



9 10 11i 1: Indanocine

A) Previous methods[15] [Shih et al. Bioorg. Med. Chem. Lett. 2000]

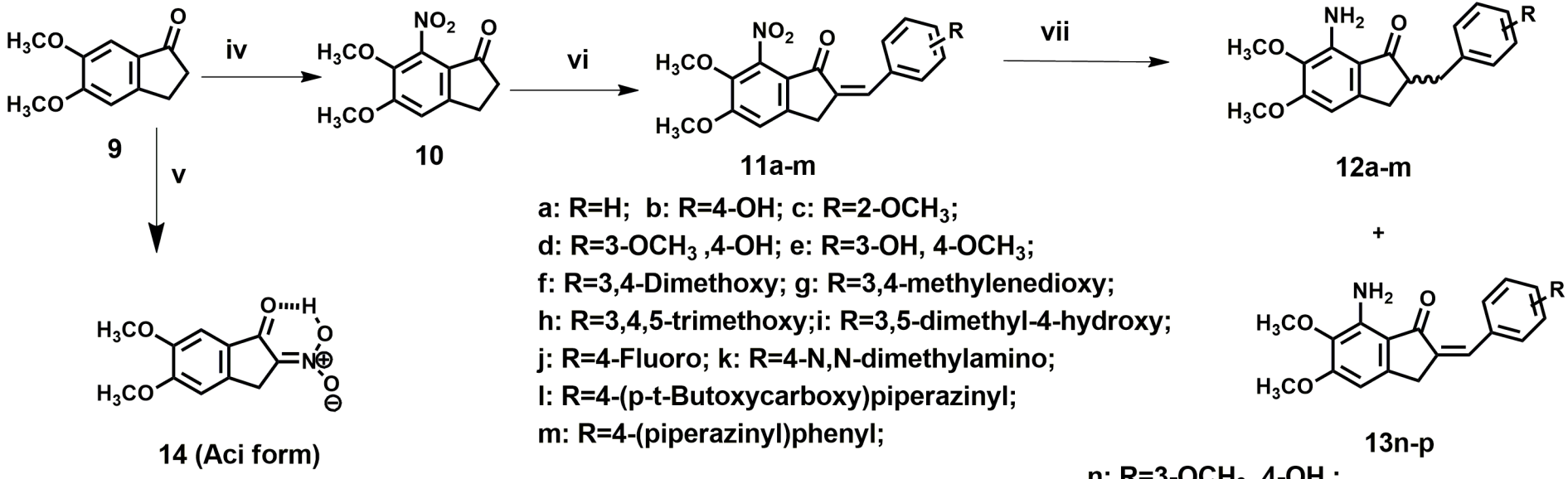
i) $NaNO_2$, TFA, 0°C→RT; ii) 3,5-dimethyl4-hydroxybenzaldehyde, 5% CH_3SO_3H in AcOH;
iii) $Na_2S_2O_4$ or Zn dust, 2%AcOH in dioxane, 100°C

Carson et al.[5] : US Patent no. 6,162,810, : ii) 3,5-dimethyl-4-hydroxybenzaldehyde,1N NaOH/EtOH, 50°C, overnight, 96% ;
iii) $NaHSO_3$, 1N NaOH/EtOH/H_2O, 80°C, 18%;
Overall yield: 17.3% (in last two steps, did not mention first step yield)

B) Our protocol (Present work)

i) Ac_2O-HNO_3, 5-10°C, 3h, 74%; ii) 3,5-dimethyl-4-hydroxybenzaldehyde, Dioxane, Conc. HCl, 110°C, 8h, 82%;
iii) 10% Pd-C, H_2, DMF, RT, 30min., 44%;
Overall yield: 26.7% (in 3 steps); 36.1% (in last two steps)

Scheme 2


iv
v
vi
vii
9
10
11a-m
12a-m
+
13n-p
14 (Aci form)
a: R=H; b: R=4-OH; c: R=2-OCH3;
d: R=3-OCH3 ,4-OH; e: R=3-OH, 4-OCH3;
f: R=3,4-Dimethoxy; g: R=3,4-methylenedioxy;
h: R=3,4,5-trimethoxy;i: R=3,5-dimethyl-4-hydroxy;
j: R=4-Fluoro; k: R=4-N,N-dimethylamino;
l: R=4-(p-t-Butoxycarboxy)piperazinyl;
m: R=4-(piperazinyl)phenyl;
n: R=3-OCH3, 4-OH,;
o: R=3,4-methylenedioxy;
p (Indanocine, 1) : R=3,5-dimethyl,4-hydroxy