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Original article

COX inhibitors Indomethacin and Sulindac derivatives as antiproliferative agents: Synthesis, biological evaluation, and mechanism investigation

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ABSTRACT

Cyclooxygenase (COX) inhibitors Indomethacin and its structural analogs Sulindac exhibit cell growth inhibition and apoptosis inducing activities in various cancer cell lines via COX independent mechanisms. In this study, the molecular structures of Indomethacin and Sulindac were used as starting scaffolds to design novel analogs and their effects on the proliferation of human cancer cells were evaluated. Compared to Indomethacin and Sulindac inhibiting cancer cell proliferation with IC_{50}s of more than 1 mM, the derivatives displayed significantly increased activities. Especially, one of the Indomethacin analogs inhibited the growth of a series of cancer cell lines with IC_{50}s around 0.5 μM–3 μM. Mechanistic investigation revealed that the new analog was in fact a tubulin inhibitor, although the parental compound Indomethacin did not show any tubulin inhibitory activity. Tubulin polymerization assay indicated this compound inhibited tubulin assembly at high concentrations, but promoted this process at low concentrations which is a very unique mechanism. The binding mode of this compound in tubulin was predicted using the molecular docking simulation.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used for the treatment of inflammatory conditions and the mechanism of the action is mainly related to the inhibition of the cyclooxygenase-2 enzyme (COX-2) [1]. In recent years, a growing body of experimental and epidemiological evidence has demonstrated that NSAIDs also display promising chemopreventive activities, especially for colorectal cancer with high COX-2 expression [2,3]. Generally, it was believed that the pharmacological basis for their anti-cancer or cancer preventive activities may involve COX-2 inhibition [4–6], because prostaglandins generated by COX-2 could promote tumor invasiveness, angiogenesis, and progression in cancers [7–9]. Inhibition of COX-2 would arrest carcinogenesis and thus prevent cancer development and regress cancer once developed. However, more compelling evidence suggests that some COX-2 independent mechanisms may be involved [10,11]. Notably, there is a lack of correlation between COX-2 inhibitory potency and anti-cancer activity of these NSAIDs [12]. Some non-COX-2 inhibitory analogs derivatized from COX-2 inhibitors still exhibit potent anti-cancer activities [13,14]. These molecules may block other cellular machineries to affect the cancer cell function, which could lead to cell growth inhibition, apoptosis or necrosis. For instance, COX-2 inhibitor Celecoxib derivative OSU-03012 inhibits growth and induces apoptosis of tumor cell via 3-phosphoinositide dependent protein kinase-1 (PDK-1) inhibition [15,16]. More recently, our laboratory identified the anti-cancer molecular target of COX-2 inhibitor nimesulide analog NSC751382 to be tubulin and heat shock protein 27 (Hsp27), which are well known targets for anti-cancer drugs development [17].

Although it is debatable about the mechanism for anti-cancer effects of COX-2 inhibitors, some NSAIDs have been evaluated as anti-cancer agents either alone or in combination with other chemotherapeutic agents in the preclinical and clinical studies [18–22]. However, using COX-2 inhibitors may be associated with cardiovascular side effects, which limit the application of COX-2 inhibitors in cancer chemotherapy [23]. Development of non-COX-2 active analogs based on known COX-2 inhibitors is an important strategy for the discovery of new anti-cancer drugs with high efficiency and less toxicity. It is feasible to completely

Abbreviation: COX, Cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; PDK, phosphoinositide-dependent kinase; Hsp27, heat shock protein 27; Wnt, "Wnt" was created from a combination of the Drosophila segment polarity gene *Wingless* and the mouse proto-oncogene *Int*-1; MAPK, mitogen activated protein kinase; PDE5, phosphodiesterase type 5; SAR, structure activity relationship; DCC, *N,N′*-dicyclohexylcarbodiimide; PyBOP, benzotriazol-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate.

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eliminate the COX-2 inhibitory activity and improve the anti-cancer potency through chemical modification of the NSAIDs with promising anti-cancer activities [13,14,16]. In our effort to develop novel anti-cancer agents, we aim at the COX inhibitor Indomethacin and its structural analog Sulindac as lead compounds (Fig. 1). This selection is based on the well-documented COX independent anti-cancer activities of these two anti-inflammatory agents [20,21,24–26], and their similar COX inhibition kinetics [27,28]. Indomethacin inhibits human colorectal cancer cell growth by inducing G1 arrest and apoptosis, which is associated with down-regulation of β-catenin and influencing the Wnt (Wnt is created from a combination of the Drosophila segment polarity gene *Wingless* and the mouse proto-oncogene *Int*-1) signaling pathway [29]. It also suppresses angiogenesis though inhibition of mitogen-activated protein kinase activity (MAPK) [30]. Sulindac sulfide is found to inhibit colon tumor cell growth via induction of apoptosis through phosphodiesterase type 5 (PDE5) inhibition [31]. However, as anti-cancer agents, Indomethacin and Sulindac (or Sulindac sulfide) are not very potent and have limited application in cancer therapy.

In this study, we used Indomethacin as lead compound and synthesized a series of derivatives through systematic modification of carboxylic acid moiety and benzoyl ring (Fig. 1). Interestingly, one of the derivatives exhibited strong antiproliferative activity against multiple cancer cell lines via interfering with tubulin polymerization. Considering the structure similarity between Indomethacin and Sulindac, we also systematically modified Sulindac to search more potent anti-cancer derivatives (Fig. 1). Sulindac analogs as anti-cancer agents have been reported before and some of them show promising antiproliferative activities [32,33]. However, the structural features which are responsible for anti-cancer effects remain unclear [32,33]. In this work, chemical modification of Sulindac still aim at carboxylic acid moiety and benzylidene ring. A series of new derivatives were synthesized and evaluated as antiproliferative agents against colon caner cells HT29 in order to further systematically interpret the structure activity relationship (SAR).

2. Results and discussion

2.1. Compound design and synthesis

Indomethacin was used as molecular scaffold to generate a diversity of derivatives in an effort to improve the anti-cancer activity. The Marnett group reported that conversion of Indomethacin to its amide derivatives can lead to some potent and selective COX-2 inhibitors [34], which may contribute to the anti-cancer activity. So, the carboxyl group of Indomethacin was initially converted to an amide bond. In the presence of the general coupling reagent such as *N*,*N*′-dicyclohexylcarbodiimide (DCC) or benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophos phate (PyBOP), Indomethacin reacted with a variety of aromatic or aliphatic amines to afford compounds **1**–**14** (Table 1). Next, 4-chlorobenzoyl group was replaced by a series of substituted benzoyl groups to give the second generation of derivatives **16**–**24** in order to investigate the effects of different substituent groups on the phenyl ring. Different from all the other Indomethacin derivatives, Compound **15** is a sulfonamide derivative which was prepared to investigate the effect of replacement of the amide bond in Indomethacin with a sulfonamide bond. The commercial available starting material 5-methoxy-2-methyl-3-indoleacetic acid reacted with the corresponding aliphatic amine to generate amide intermediate, which subsequently reacted with benzenesulfonyl chloride or substituted benzoyl chloride in the presence of sodium hydride (Scheme 1). The similar compound design strategy was also applied to generate Sulindac analogs. The amide derivatives of Sulindac **25**–**35** were synthesized using PyBOP mediated coupling of Sulindac with a diversity of amines (Table 2). Moreover, in order to evaluate the effect of variation of the substitutions on the benzylidene ring, compounds **36**–**41** were synthesized. (5-Fluoro-2-methyl-1*H*-inden-3-yl) acetic acid coupled with the corresponding amine followed by condensation with appropriate substituted benzaldehyde (Scheme 2). All the products were purified by recrystallization or flash column chromatograph.

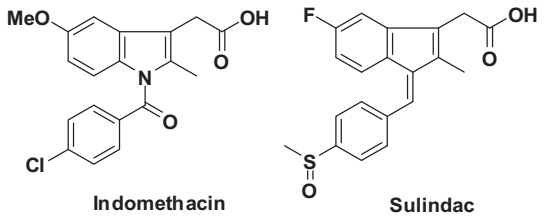


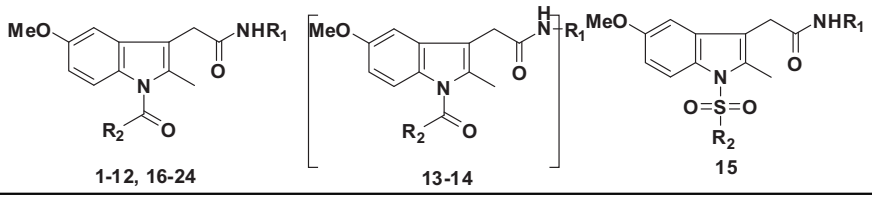
Fig. 1. Chemical structures of Indomethacin and Sulindac.

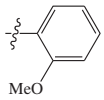
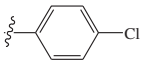
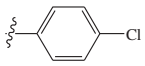
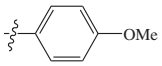
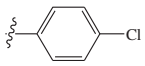
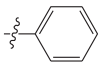
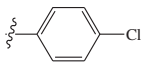
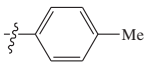
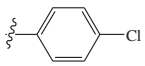
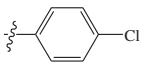
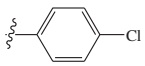
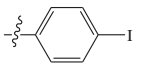
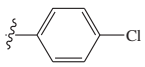
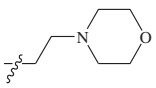
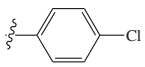
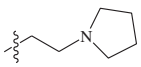
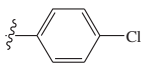
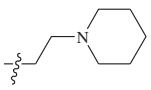
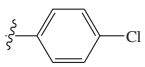
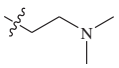
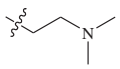
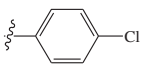
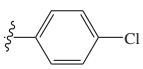
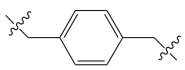
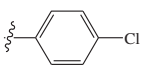
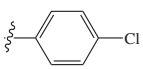
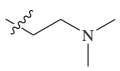
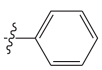
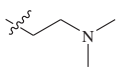
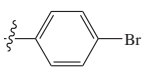
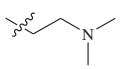
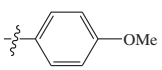
2.2. Cell growth inhibition of Indomethacin derivatives

All the Indomethacin derivatives were screened for their anti-proliferative activities against colon cancer cell line HT29 and the results are summarized in Table 1. Aromatic amide derivatives of Indomethacin **1**–**7** displayed different anti-proliferative activity and IC_{50} values ranged from 52 μM to 1314 μM. Compared with Indomethacin, phenyl amide **4** exhibited improved activity with IC_{50} of 219 μM. *Ortho*- or *meta*- or *para*-methoxy substitution on phenyl (compounds **1**–**3**) decreased the inhibitory activity compared with phenyl amide **4**, while *para*-chlorophenyl amide (**6**), *para*-iodophenyl amide (**7**), and *para*-methylphenyl amide (**5**) exhibited slightly better inhibitory potency. This result suggests that the substituent groups such as chloro, iodo, and methyl in the benzamide moiety are tolerated better than methoxy group. With the exception of compound **12**, all the aliphatic amide derivatives (**8**–**11**) exhibited better cell growth inhibitory activity than Indomethacin. Most notably, 2-morpholinoethyl amide **8** is about 45-fold more active with IC_{50} of 23 μM. Replacement of oxygen in 2-morpholinoethyl moiety of **8** with CH_2 to give compound **10** resulted in a 12-fold loss in the potency of cell growth inhibition. 2-(pyrrolidin-1-yl) ethyl amide derivative **9** has an IC_{50} of 38 μM, slightly less potent than 2-morpholinoethyl amide **8**. Compound **11** is less active than compounds **8** and **9**, which may be due to the different properties of aliphatic moieties. The terminal rings of 2-morpholinoethyl and 2-(pyrrolidin-1-yl) ethyl in compounds **8** and **9** are more bulky and less rigid than dimethylamino group in compound **11**. Compounds **13** and **14** are dimmers generated from coupling of two molecules of Indomethacin with one molecule of diamine. IC_{50} of compound **14** was determined to be 110 μM and the biological evaluation of compound **13** was not completed due to its poor solubility in cell culture medium. Through this preliminary study, we identified two most active compounds, **8** and **9**, which represented early benchmark compounds. We also considered further optimization of compound **11**. Compound **11** has superior solubility than compounds **8** and **9**, probably due to the hydrophilic property of its terminal dimethylamino group. Moreover, the smaller size and more flexibility of the terminal dimethylamino group compared to the rings (morpholine and pyrrolidine) can be a benefit for ligand binding.

Keeping the 2-dimethylaminoethyl amide moiety and varying substitutions on the benzoyl ring, including 4-bromo, 4-methoxy, 3,4-dimethoxy, and 3,4,5-trimethoxy, yielded derivatives that are much more active than compound **11**. Replacement of 4-

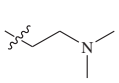
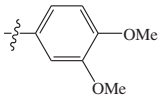
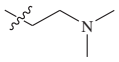
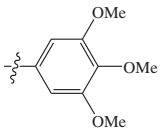
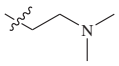
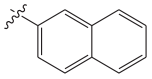
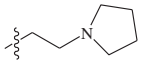
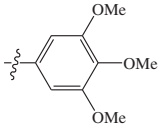
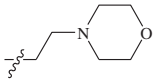
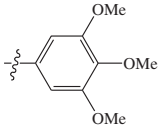
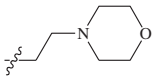
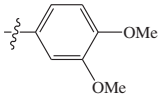
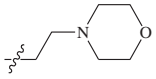
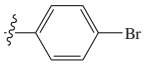
Table 1
IC_{50} of inhibition of HT29 colon cancer cell growth by Indomethacin derivatives.



Compound	R_1	R_2	$IC_{50}/\mu M$
Indomethacin			1052 ± 161
1			1124 ± 463
2			1190 ± 354
3			1314 ± 519
4			219 ± 187
5			68 ± 33
6			161 ± 104
7			52 ± 24
8			23 ± 13
9			38 ± 14
10			277 ± 220
11			170 ± 94
12	$(CH_2)_5CH_3$		1334 ± 533
13			ND[a]
14	$(CH_2)_5$		110 ± 85
15			7.7 ± 2.6
16			13.5 ± 4.9
17			17.8 ± 8.3

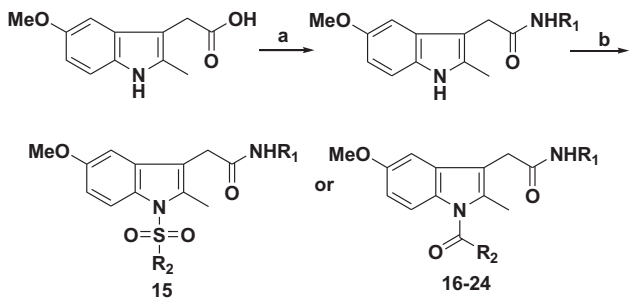
(*continued on next page*)

Table 1 (*continued*)

Compound	R_1	R_2	IC_{50}/μM
18			44 ± 15
19			2.7 ± 0.8
20			29 ± 13
21			34 ± 13
22			611 ± 252
23			ND[a]
24			ND[a]

[a] Not determined due to poor solubility of the compound in media.

chlorobenzoyl ring in compound **11** with a larger aromatic ring system such as 2-naphthoyl, gave derivative **20** with a significant increase in potency. In the series of 2-dimethylaminoethyl amide derivatives, 3,4,5-trimethoxybenzoyl derivative **19** was found to be the most potent inhibitor with IC_{50} of 2.71 μM. Moreover, replacement of 2-dimethylaminoethyl group in compound **19** with 2-morpholinoethyl (**22**) or 2-(pyrrolidin-1-yl) ethyl (**21**) resulted in a significant decrease in potency. Compounds **23** and **24** were not evaluated due to their poor solubility in cell culture medium. Phenyl sulfonyl derivative **15** does not belong to the same series. Unexpectedly, it showed very promising activity with IC_{50} of 7.7 μM and could be the first term of a new series.



15 R_1= 2-(dimethylamino)ethyl; R_2= phenyl
16 R_1= 2-(dimethylamino)ethyl; R_2= 4-bromophenyl
17 R_1= 2-(dimethylamino)ethyl; R_2= 4-methoxyphenyl
18 R_1= 2-(dimethylamino)ethyl; R_2= 3, 4-dimethoxyphenyl
19 R_1= 2-(dimethylamino)ethyl; R_2= 3,4,5-trimethoxyphenyl
20 R_1= 2-(dimethylamino)ethyl; R_2= 2-naphthyl
21 R_1= 2-(pyrrolidin-1-yl)ethyl; R_2= 3,4,5-trimethoxyphenyl
22 R_1= 2-morpholinoethyl; R_2= 3,4,5-trimethoxyphenyl
23 R_1= 2-morpholinoethyl; R_2= 3, 4-dimethoxyphenyl
24 R_1= 2-morpholinoethyl; R_2= 4-bromophenyl

a) R_1NH_2, PyBOP, Et_3N, DMF; b) R_2SO_2Cl or R_2COCl, NaH, DMF

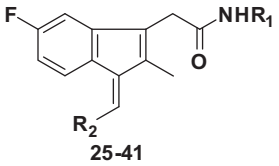
Scheme 1. Synthesis of Indomethacin derivatives **15**–**24**.

These results reveal some interesting SAR for anti-cell proliferative activity by Indomethacin derivatives. Changing the carboxylate moiety to bulky alkyl amines via amidation benefits the cell growth inhibitory activity. Substituents on the benzoyl ring also significantly affect the potency of compounds. The preference for 3,4,5-trimethoxy on the benzoyl ring was observed.

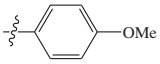
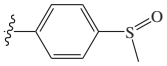
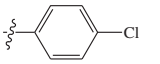
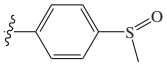
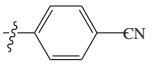
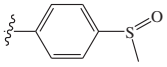
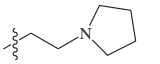
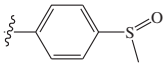
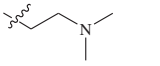
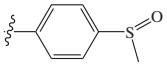
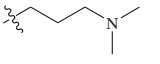
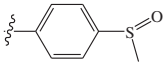
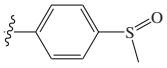
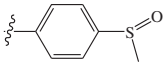
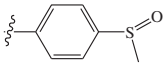
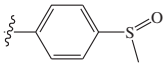
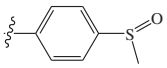
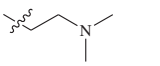
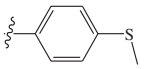
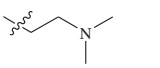
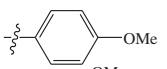
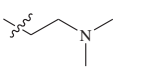
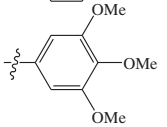
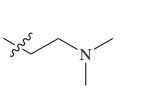
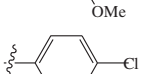
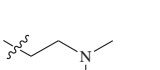
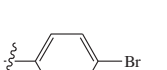
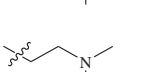
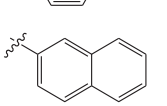
2.3. Cell growth inhibition of Sulindac derivatives

Three representative aromatic amide derivatives of Sulindac **25**–**27** were evaluated initially. As shown in Table 2, these compounds did not show an increase in cell growth inhibition potency compared to Sulindac and no more aromatic amide derivatives were further synthesized and evaluated here. The amidation of carboxylate group in Sulindac by a series of alkyl amines yielded a diversity of alkyl amide derivatives with different inhibitory activity. Among this series of Sulindac derivative (**28**–**35**) investigated, 2-(pyrrolidin-1-yl) ethyl, 2-dimethylaminoethyl, and n-hexyl amide derivatives (**28**, **29** and **32**) are the most active

Table 2
IC_{50} of inhibition of HT29 colon cancer cell growth by Sulindac derivatives.



25-41

Compound	R_1	R_2	$IC_{50}/\mu M$
Sulindac			1729 ± 679
25			2539 ± 643
26			1697 ± 519
27			2580 ± 956
28			52 ± 28
29			47 ± 23
30			465 ± 196
31	$(CH_2)_3CH_3$		124 ± 48
32	$(CH_2)_5CH_3$		53 ± 26
33	$(CH_2)_7CH_3$		307 ± 112
34	$CH(CH_3)(CH_2)_4CH_3$		2142 ± 917
35	$CH_2CH_2CH(CH_3)_2$		1426 ± 692
36			11.3 ± 3.5
37			20 ± 7
38			140 ± 80
39			12.4 ± 6.4
40			20 ± 7
41			271 ± 135

compounds with IC_{50}s around 50 μM. Extension of the carbon chain in compound **29** to give compound **30** leaded to a 10-fold loss of activity. The linear six carbon chain in *n*-hexyl amide derivative **32** appeared to be optimal. Either extension or shortening of the carbon chain (compounds **31** or **33**) leaded to a significant decrease in potency. The alkyl amide derivatives with branched carbon chains such as compounds **34** and **35** showed weaker inhibitory activities compared to the linear alkyl amide.

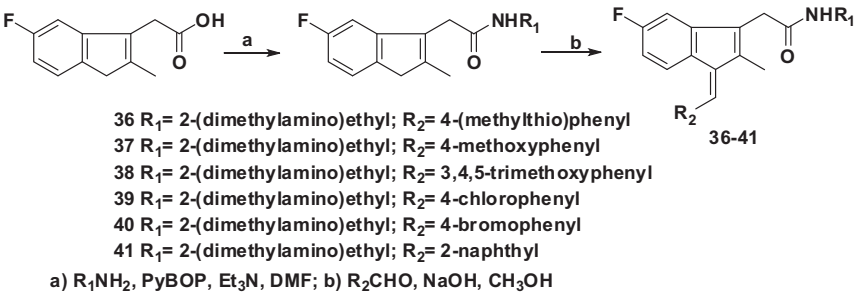
Based on the most active compound **29**, another series of compounds were generated by retaining the alkyl amide moiety and replacing the 4-methylsulfinyl benzylidene with other substituted aromatic groups. Various substitutions on 4-position of benzylidene ring, including methylthio, methoxy, chloro, and bromo, led to derivatives (**36**, **37**, **39**, and **40**) that are about 2–4 fold more active than compound **29**. In contrast, bulky aromatic groups like 3,4,5-trimethyloxybenzylidene (**38**) and 2-naphthyl (**41**) replaced 4-methylsulfinyl benzylidene in compound **29**, resulted in a significant decrease in potency. It seems that the large aromatic systems are not favored in this moiety. Overall, it seems that Indomethacin is a better scaffold to generate promising anti-cancer derivatives compared with Sulindac.

2.4. Effects of the potent new derivatives on the growth of multiple cancer cell lines

To further investigate the cell growth inhibition potency and selectivity, the effect of three most active Indomethacin derivatives **15**, **16** and **19** against a panel of five additional tumor cell lines and one normal human fibroblast cell line were evaluated and the results are summarized in Table 3. In general, all the compounds are more active against the tumor cell lines than the normal fibroblast cell line IMR90. Compound **19** was also found to be the most active one to strongly inhibit the growth of these tumor cells including SKBR-3, H292, H522, MDA468, and MCF-7, with IC_{50} values of 4.63, 0.51, 3.00, 2.87, and 0.90 μM respectively. It suggests that compound **19** may exhibit activity through a ubiquitous biological molecule which is important for cell proliferation in all the cancer cell lines.

2.5. The new analogs interfere with tubulin polymerization

Our SAR analysis revealed that the 3,4,5-trimethylbenzoyl group in compound **19** played an important role in the cell growth inhibition. Since numerous compounds incorporating the structure of 3,4,5-trimethylphenyl display potent antiproliferative activity through the mechanism of tubulin polymerization inhibition [35,36], we anticipated that our compounds especially compound **19** may also interact with tubulin. In order to confirm this speculation, we evaluated compound **15**, **16**, and **19** with an *in vitro* tubulin polymerization assay. As shown in Fig. 2 (A), all three compound at 25 μM inhibited tubulin polymerization and the order of potency was **19** > **15** > **16**, which appeared to be consistent with the potency of cell growth inhibition. It seems that the methoxy group contributed to the tubulin interfering activity of the compounds, since compound **19** with tri-methoxy groups exhibited the best potency against tubulin polymerization. Fig. 2 (B) shows that compound **19** affected the polymerization of tubulin in a dose dependent manner. In 10 min, **19** inhibited tubulin polymerization by 20% at 8 μM and 56% at 10 μM, as compared to DMSO. Increasing inhibitor concentration to 25 μM did not cause more inhibition, which suggested that the binding sites of tubulin might be saturated by the inhibitor. However, **19** at the lower concentrations (2 and 5 μM) appreciably promoted tubulin polymerization, similar to the effect caused by Taxol. This is a very unique phenomenon of the new tubulin inhibitors. It suggests that compound **19** may have



Scheme 2. Synthesis of Sulindac derivatives **36**–**41**.

multiple binding sites on tubulin, and the various binding modes result in different activities to tubulin.

2.6. Molecular docking analysis

Compound **19** is a diaryl system with a tri-methoxyphenyl moiety that is the common feature of numerous colchicine site inhibitors [36]. We anticipated that compound **19** may target the colchicine binding site in tubulin. In order to elucidate the interaction between compound **19** and tubulin, we conducted a docking simulation of compound **19** into the colchicine binding site. The results are shown in Fig. 3. The docked structure of compound **19** was found be partly overlapped with the crystal structure of colchicine taken as reference (Fig. 3A). Compound **19** is located in the bind site with 3,4,5-trimethoxyphenyl group positioned at the hydrophobic pocket defined by Ala 250, Cys 241, Val 238, Tyr 202, Ile 378, and Leu 255. The carbonyl group on the indole nitrogen of compound **19** can form a hydrogen bond with the main chain of Leu 255. Another potential weak hydrogen bond may be established between the 4-methoxy group in trimethoxyphenyl moiety of compound **19** and the mercapto group of Cys 241 (Fig. 3B). Overall, this docking result suggests that tri-methoxyphenyl moiety plays an important role in the binding of compound **19** at colchicine-binding domain of tubulin.

On the other hand, assembly promoting properties of compound **19** at the lower concentrations prompted us to consider the other possible binding site for this compound. To date, most tubulin assembly inducing compounds bind at the taxoid site except laulimalide and peloruside A [37]. The taxoid site is well characterized with the crystal structure of the α,β-tubulin heterodimer complexed with Taxol [38]. In order to evaluate the binding affinity of compound **19** at the taxoid site, we performed a docking simulation of compound **19** into this site (Fig. 3C). Compound **19** fits well in the binding site, although it does not share any similar structural features with most assembly inducing compounds which bind at the taxoid site. As shown in Fig. 3D, three potential hydrogen bonds can be formed between compound **19** and the taxoid site. 3-Methoxy group in trimethoxyphenyl moiety of compound **19** may serve as hydrogen bond acceptor to the amino group of the main chain of Thr276. Another hydrogen bond may be formed between 5-methoxy group in indole ring and hydroxyl group of Ser236. Moreover, the aliphatic amide group of compound **19** also can form a hydrogen bond with the imidazole of His229. It should be noted that the *N*,*N*-dimethylamino group in compound **19** can become positively charged by accepting one proton from medium and there may be electrostatic interaction between the charged dimethylamino group and the carboxyl groups in Asp26 and Glu22.

3. Conclusion

In this work, Indomethacin and Sulindac analogs were generated by systematic modification of carboxylic acid moiety and benzoyl ring or benzylidene ring. The antiproliferative activity of all the derivatives was evaluated against colon cancer cells HT29. We found that for both Indomethacin and Sulindac the amidation of the carboxylate moiety by some alkyl amines such as 2-(pyrrolidin-1-yl) ethyl amine and 2-dimethylaminoethylamine resulted in a significant increase in potency. Moreover, some alkyl amide derivatives of Indomethacin displayed similar activities to the same alkyl amide derivatives of Sulindac. For example, 2-(pyrrolidin-1-yl) ethyl amide derivatives **9** and **28** exhibited the same level of activity as evidenced by similar IC_{50} values of 38 μM and 52 μM respectively. It is also same with 2-dimethylaminoethyl amide derivatives **11** and **29**. The similar SAR of the amide moiety may be attributed to the similarity of chemical structures of Sulindac and Indomethacin. Retaining the optimal alkyl amide moiety and further modification of benzoyl ring in Indomethacin and benzylidene in Sulindac gave several pairs of compounds with chemical structures similar to each other like **16** vs **40**, **17** vs **37**, **20** vs **41**, **19** vs **38**. For each pair of compounds, the major difference is indole amide bond in the former and benzylidene double bond in the latter. Compounds**16** and **40** exhibited the same level of activity. Also, compound **17** is nearly as potent as **37**. In contrast, compound **20** and **41** exhibited significant different activities and the former is nearly 10-fold more potent than the latter. The great difference in activities was also observed for compounds **19** and **38**. It appears that the bulky aromatic rings 2-naphthyl and 3,4,5-trimethyloxyphenyl are more favored in the Indomethacin analogs **20** and **19** than in the corresponding Sulindac analogs **41** and **38**. It is possible that in the compounds **20** and **19**, 2-naphthyl and 3,4,5-trimethyloxyphenyl may adopt a more favorable binding conformation by rotation of the amide bond.

Of all the Indomethacin and Sulindac derivatives investigated here, Indomethacin analog **19** displayed the most potent antiproliferative activity against HT29 color cancer cells with an IC_{50} of 2.71 μM. The similar growth inhibition was also observed for this compound in multiple cancer cell lines. The tubulin polymerization

Table 3
Antiproliferative activity of Indomethacin derivatives **15**, **16** and **19** against different cell lines.

Compound	IC_{50}/μM					
	SKBR-3	H292	H522	MDA468	MCF-7	IMR90
15	6.39 ± 7.37	4.85 ± 2.55	14.09 ± 6.06	15.91 ± 15.27	4.88 ± 1.51	18.68 ± 9.51
16	4.81 ± 2.31	3.47 ± 1.24	10.61 ± 5.95	2.91 ± 1.83	9.53 ± 2.84	41.72 ± 44.44
19	4.63 ± 1.54	0.51 ± 0.42	3.00 ± 2.55	2.87 ± 2.95	0.90 ± 0.39	17.19 ± 9.46

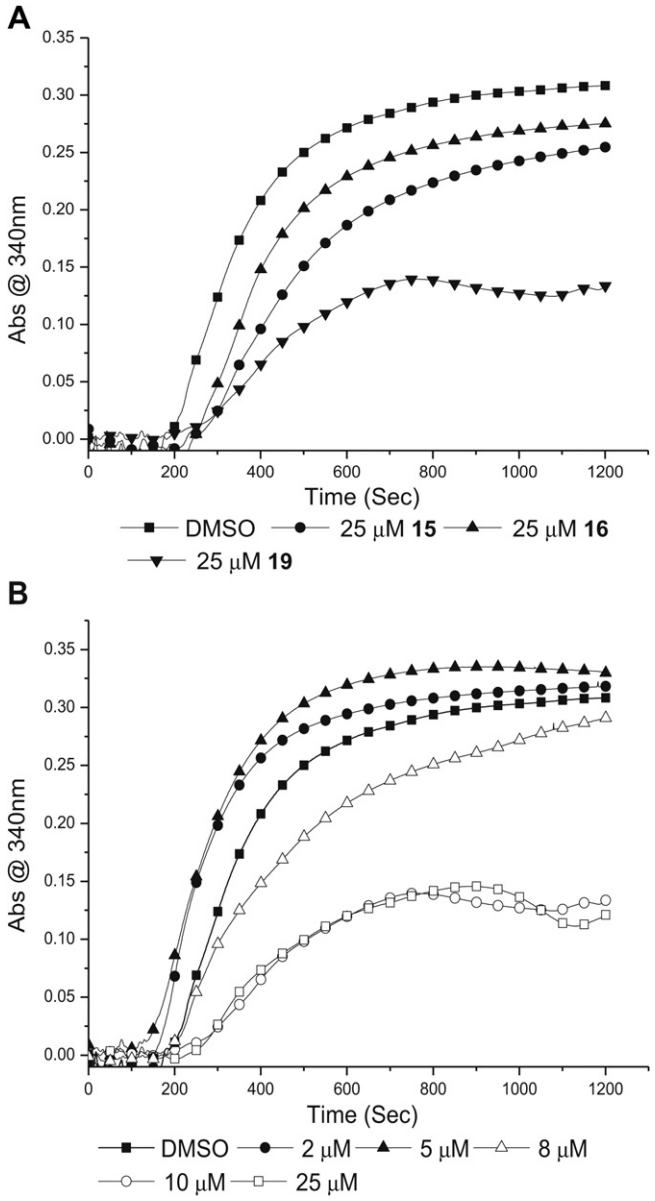


Fig. 2. (A) Tubulin polymerization in the presence of compounds **15**, **16**, and **19** respectively; (B) Tubulin polymerization in the presence of different concentrations of compound **19**.

assay indicated that compound **19** interferes with tubulin polymerization. Different from most mitotic inhibitors, compound **19** promoted tubulin assembly at low concentrations and inhibited this process at higher concentrations. Based on the result from molecular docking simulation, compound **19** is capable of binding at the colchicine site and taxoid site. The estimated lowest free energies for binding at the cochinchine site and taxoid site are −10.43 and −8.44 kcal/mol, respectively. Both binding modes can be stabilized by hydrogen bonds or hydrophobic interaction. Further optimization of compound **19** with the aid of molecular modeling study to generate more potent derivatives is currently underway.

4. Experimental section

4.1. Chemistry

Chemicals were commercially available and used as received without further purification unless otherwise noted. Moisture sensitive reactions were carried out under a dry argon atmosphere in flame-dried glassware. Thin layer chromatography was performed on silica gel TLC plates with fluorescence indicator 254 nm (Fluka). Flash column chromatography was performed using silica gel 60 Å (BDH, 40–63 μM). Mass spectra were obtained on the ABI QStar Electrospray mass spectrometer at Cleveland State University MS facility Center. All the NMR spectra were recorded on a Varian 400 MHz spectrometer (^{13}C NMR at 100 MHz) using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) for ^{1}H NMR spectra were reported in parts per million to residual solvent protons. The IR spectra were obtained on a Bruker ALPHA FT-IR spectrometer with ATR module.

Reversed-phase HPLC analysis of compounds **15**, **16**, and **19**, were conducted on Beckman HPLC system with AutoSampler. The chromatographic separation was performed on a C18 column (2.0 mm × 150 mm, 5 μm) from Phenomenex (Torrance, CA, USA). The mobile phase of 80% acetonitrile and 20% water was employed for isocratic elution with a flow rate of 0.2 ml/min. The injection volume was 20 μl and the UV detector was set up at 260 and 320 nm.

4.1.1. General procedure for preparation of Indomethacin derivatives (**1**–**14**)

Method 1: to a solution of Indomethacin (300 mg, 0.84 mmol), the corresponding amine (0.92 mmol), dicyclohexylcarbodiimide (192 mg, 0.93 mmol) in dichloromethane was added 4-dimethylaminopyridine (10 mg, 0.081 mmol) and stirred at room temperature for 2 h. The insoluble solid was filtered and the filtrate was concentrated under vacuum. The residue was diluted with water and extracted using dichloromethane. The organic layer was washed with brine and then concentrated under vacuum. The crude product was purified by recrystallization to yield the desired product.

Method 2: to the solution of Indomethacin (100 mg, 0.28 mmol), the corresponding amine (0.28 mmol), and PyBOP (145.7 mg, 0.28 mmol) in anhydrous dimethylformamide was added triethylamine (56.5 mg, 0.56 mmol) and the mixture was stirred at room temperature for 2 h. Then saturated sodium chloride solution was added. The reaction mixture was extracted using ethyl acetate (3 × 50 ml). The combined organic layers were washed successively with water, 5% aqueous sodium bicarbonate, and then concentrated under vacuum. The obtained crude product was purified by recrystallization or silica gel column chromatography.

*4.1.1.1. N-(2-Methoxyphenyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**1**).* The method 1 and *o*-anisidine were used; white solid, yield 44%; ^{1}H NMR ($CDCl_3$) δ 8.32 (1H, dd, J = 8.0, 1.6 Hz), 8.06 (1H, br), 7.69 (2H, m), 7.50 (2H, m), 6.99 (3H, m), 6.88 (1H, d, J = 9.2 Hz), 6.78 (1H, dd, J = 8, 1.2 Hz), 6.71 (1H, dd, J = 8.8, 2.8 Hz), 3.82 (5H, s), 3.633 (3H, s), 2.46 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.17, 168.53, 156.27, 150.10, 138.32, 136.08, 134.85, 131.84, 131.49, 130.98, 129.73, 127.88, 125.07, 122.32, 120.90, 115.26, 114.93, 111.99, 111.73, 102.66, 56.33, 56.08, 32.53, 14.02; ESI-MS calculated for ($C_{26}H_{24}ClN_2O_4$) $[M + H]^+$: 463.14, found: 463.26.

*4.1.1.2. N-(3-Methoxyphenyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**2**).* The method 1 and *m*-anisidine were used; white solid, yield 60%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, m), 7.50 (2H, m), 7.17 (2H, m), 6.94 (1H, d, J = 2.4 Hz), 6.88 (1H, d, J = 9.2 Hz), 6.82 (1H, d, J = 8 Hz), 6.72 (1H, dd, J = 9.2, 2.4 Hz), 6.65 (1H, dd, J = 8, 2.8 Hz) 3.81 (3H, s), 3.80 (2H, s), 3.78 (3H, s), 2.45 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.21, 168.53, 160.17, 156.23, 141.00, 138.28, 136.08, 134.87, 131.84, 131.57, 130.93, 130.20, 129.73, 115.26, 114.76, 112.09, 111.79, 109.44, 105.55, 102.65, 56.09, 55.60, 32.73, 14.09; ESI-MS calculated for ($C_{26}H_{24}ClN_2O_4$) $[M + H]^+$: 463.14, found: 463.26.

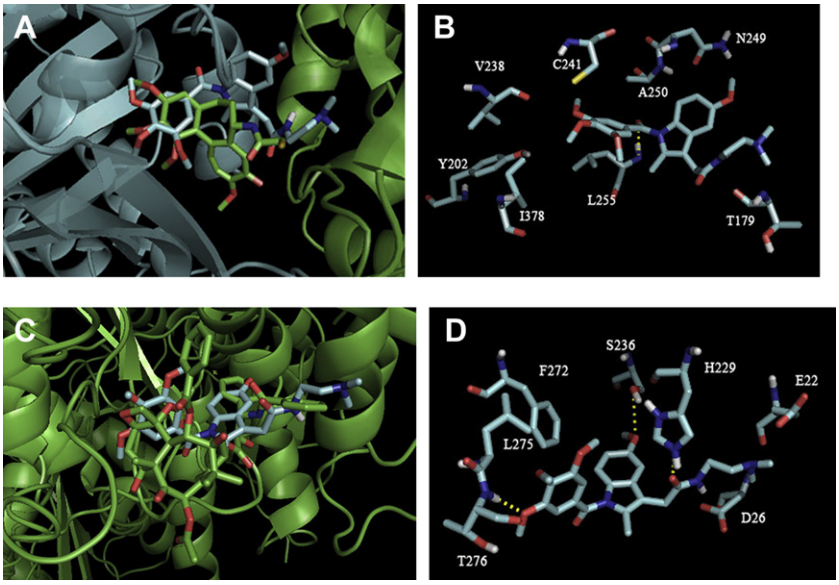


Fig. 3. The molecular docking simulation of the interaction between compound **19** and tubulin. (A) Superposition of crystal structure-based binding mode of colchicine (green) and the docked conformation of compound **19** (cyan) at the colchicine binding site. (B) Proposed binding mode of compound **19** at the colchicine binding site. (C) Superposition of crystal structure-based binding mode of Taxol (green) and the docked conformation of compound **19** (cyan) at the taxoid binding site. (D) Proposed binding mode of compound **19** at the taxoid binding site. The tubulin polypeptide backbones are shown as ribbons. Only important active site amino acids around compound **19** are shown for clarity. The dash lines indicate potential intermolecular hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*4.1.1.3. N-(4-Methoxyphenyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**3**).* The method 1 and *p*-anisidine were used; white solid, yield 57%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, m), 7.50 (2H, m), 7.27 (2H, m), 7.18 (1H, br), 6.95 (1H, d, $J = 2.4$ Hz), 6.87 (1H, d, $J = 9.2$ Hz), 6.81 (2H, m), 6.72 (1H, dd, $J = 9.2$, 2.4 Hz), 3.81 (3H, s), 3.79 (2H,s), 3.77 (3H, s), 2.45 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.66, 168.53, 156.23, 155.89, 138.26, 136.01, 134.89, 132.98, 131.83, 131.60, 130.94, 129.72, 121.49, 115.25, 114.97, 114.48, 111.81, 102.68, 56.10, 55.78, 32.60, 14.11; ESI-MS calculated for ($C_{26}H_{24}ClN_2O_4$) $[M + H]^+$: 463.14, found: 463.26.

*4.1.1.4. N-Phenyl-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**4**).* The method 1 and aniline were used; white solid, yield 25.5%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, d, $J = 8.4$ Hz), 7.50 (2H, d, $J = 8.4$ Hz), 7.38 (2H, d, $J = 7.6$ Hz), 7.28 (3H, m), 7.09 (1H, m), 6.95 (1H, d, $J = 2.4$ Hz), 6.88 (1H, d, $J = 9.2$ Hz), 6.72 (1H, dd, $J = 8.8$, 2 Hz), 3.81 (5H, s), 2.46 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.15, 168.53, 156.25, 139.82, 138.27, 136.07, 134.88, 131.84, 131.58, 130.93, 129.73, 129.40, 123.96, 119.90, 115.26, 114.81, 111.82, 102.64, 56.09, 32.70, 14.10; ESI-MS calculated for ($C_{25}H_{22}ClN_2O_3$) $[M + H]^+$: 433.13, found: 433.24.

*4.1.1.5. N-(p-Tolyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**5**).* The method 1 and *p*-toluidine were used; white solid, yield 24.6%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, m), 7.50 (2H, m), 7.26 (2H, d, $J = 8.4$ Hz), 7.21 (1H, br), 7.08 (2H, d, $J = 8.4$ Hz), 6.94 (1H, d, $J = 2.4$ Hz), 6.88 (1H, d, $J = 9.2$ Hz), 6.72 (1H, dd, $J = 8.8$, 2.4 Hz), 3.81 (3H, s), 3.80 (2H, s), 2.45 (3H, s), 2.29 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.89, 168.53, 156.22, 138.26, 137.30, 136.03, 134.89, 132.86, 131.84, 131.58, 130.93, 129.77, 129.73, 119.93, 115.26, 114.88, 111.82, 102.64, 56.09, 32.68, 21.10, 14.10; ESI-MS calculated for ($C_{25}H_{24}ClN_2O_3$) $[M + H]^+$: 447.15, found: 447.26.

*4.1.1.6. N-(4-Chlorophenyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**6**).* The method 1 and 4-chloroaniline were used; white solid, yield 72%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, m), 7.50 (2H, m), 7.34 (2H, m), 7.24 (3H, m), 6.93 (1H, d, $J = 2.4$ Hz), 6.87 (1H, d, $J = 8.4$ Hz), 6.72 (1H, dd, $J = 9.2$, 2.4 Hz), 3.81 (5H, s), 2.46 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.31, 168.53, 156.24, 138.74, 138.29, 136.14, 134.85, 131.84, 131.54, 130.93, 129.73, 129.32, 127.52, 121.43, 115.27, 114.57, 111.84, 102.59, 56.09, 32.67, 14.09; ESI-MS calculated for ($C_{25}H_{21}Cl_2N_2O_3$) $[M + H]^+$: 467.09, found: 467.21.

*4.1.1.7. N-(4-Iodophenyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**7**).* The method 1 and 4-iodoaniline were used; white solid, yield 20%; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, d, $J = 8.4$ Hz), 7.51 (7H, m), 6.91 (1H, s), 6.86 (1H, d, $J = 9.2$ Hz), 6.73 (1H, d, $J = 9.2$ Hz), 3.84 (2H, s), 3.81 (3H, s), 2.47 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.33, 168.53, 156.24, 139.62, 138.29, 138.06, 136.13, 134.85, 131.84, 131.52, 130.92, 129.74, 122.08, 115.28, 114.56, 111.84, 102.59, 87.44, 56.10, 32.73, 14.10; ESI-MS calculated for ($C_{25}H_{21}ClIN_2O_3$) $[M + H]^+$: 559.03, found. 559.18.

*4.1.1.8. N-(2-Morpholinoethyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**8**).* The method 2 and 4-(2-aminoethyl)morpholine were used; white solid, yield 74%; ^{1}H NMR ($CDCl_3$) δ 7.68 (2H, m), 7.50 (2H, m), 6.89 (1H, d, $J = 2.8$ Hz), 6.83 (1H, d, $J = 9.2$ Hz), 6.69 (1H, dd, $J = 9.2$, 2.8 Hz), 6.27 (1H, br), 3.81 (3H, s), 3.65 (2H, s), 3.36 (4H, br), 3.29 (2H, m), 2.42 (3H, s), 2.35 (2H, t, $J = 6$ Hz), 2.23 (4H, m); ^{13}C NMR (DMSO-d_6) δ 169.97, 168.51, 156.21, 138.24, 135.83, 134.92, 131.81, 131.54, 130.96, 129.71, 115.20, 115.01, 111.80, 102.65, 66.77, 58.02, 56.09, 53.89, 36.64, 31.86, 14.05; ESI-MS calculated for ($C_{25}H_{29}ClN_3O_4$) $[M + H]^+$: 470.18, found: 470.30.

*4.1.1.9. N-(2-(Pyrrolidin-1-yl)ethyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**9**).* The method 2 and 1-(2-aminoethyl)pyrrolidine were used; white solid, yield 94%; ^{1}H NMR ($CDCl_3$) δ 7.66 (2H, m), 7.48 (2H, m), 6.91 (1H, d, $J = 2.4$ Hz), 6.86 (1H, d, $J = 8.8$ Hz), 6.69 (1H, dd, $J = 9.2$, 2.4 Hz), 6.35 (1H, br), 3.82 (3H, s), 3.63 (2H, s), 3.30 (2H, m), 2.49 (2H, t, $J = 6$ Hz), 2.39 (3H, s), 2.34 (4H, m), 1.59 (4H, m); ^{13}C NMR (DMSO-d_6) δ 169.96, 168.50, 156.21, 138.23, 135.77, 134.93, 131.80, 131.56, 130.94, 129.69, 115.18, 115.10, 111.85, 102.59, 56.06, 55.57, 54.26, 38.80, 31.82, 23.75, 14.03;

ESI-MS calculated for ($C_{25}H_{29}ClN_3O_3$) $[M + H]^+$: 454.19, found: 454.31.

*4.1.1.10. N-(2-(Piperidin-1-yl)ethyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**10**).* The method 2 and 1-(2-aminoethyl)piperidine were used; white solid, yield 88%; ^{1}H NMR ($CDCl_3$) δ 7.67 (2H, m), 7.48 (2H, m), 6.90 (1H, d, $J = 2.4$ Hz), 6.85 (1H, d, $J = 8.8$ Hz), 6.68 (1H, dd, $J = 9.2$, 2.8 Hz), 6.39 (1H, br), 3.82 (3H, s), 3.64 (2H, s), 3.26 (2H, m) 2.40 (3H, s), 2.30 (2H, t, $J = 6$ Hz), 2.18 (4H, br), 1.25 (6H, m); ^{13}C NMR (DMSO-d_6) δ 169.90, 168.47, 156.24, 138.26, 135.78, 134.92, 131.78, 131.55, 130.96, 129.67, 115.18, 115.04, 111.84, 102.56, 58.34, 56.05, 54.68, 37.07, 31.86, 26.15, 24.67, 14.04; ESI-MS calculated for ($C_{26}H_{31}ClN_3O_3$) $[M + H]^+$: 468.21, found: 468.33.

*4.1.1.11. N-(2-Dimethylaminoethyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**11**).* The method 2 and 2-(dimethylamino)ethylamine were used; white powder, yield 69%; ^{1}H NMR ($CDCl_3$) δ 7.66 (2H, m), 7.48 (2H, m), 6.91 (1H, d, $J = 2.4$ Hz) 6.87 (1H, d, $J = 8.8$ Hz), 6.69 (1H, dd, $J = 9.2$, 2.8 Hz), 6.21 (1H, br), 3.83 (3H, s), 3.63 (2H, s), 3.28 (2H, m), 2.38 (3H, s), 2.31 (2H, t, $J = 6$ Hz), 2.90 (6H, s); ^{13}C NMR (DMSO-d_6) δ 170.00, 168.48, 156.22, 138.24, 135.75, 134.92, 131.79, 131.59, 130.96, 129.67, 115.18, 115.14, 111.86, 102.61, 58.92, 56.04, 45.82, 37.57, 31.82, 14.04; ESI-MS calculated for ($C_{23}H_{27}ClN_3O_3$) $[M + H]^+$: 428.17, found: 428.28.

*4.1.1.12. N-(n-Hexyl)-1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**12**).* The method 2 and *n*-hexylamine were used; white solid, yield 57%; ^{1}H NMR ($CDCl_3$) δ 7.67 (2H, m), 7.49 (2H, m), 6.87 (2H, m), 6.70 (1H, dd, $J = 8.4$, 2.8 Hz), 5.57 (1H, br), 3.82 (3H, s), 3.64 (2H, s), 3.19 (2H, m), 2.39 (3H, s), 1.39 (2H, m), 1.19 (6H, m), 0.83 (3H, t, $J = 6.8$); ^{13}C NMR (DMSO-d_6) δ 169.88, 168.52, 156.21, 138.22, 135.72, 134.95, 131.80, 131.56, 130.93, 129.69, 115.20, 115.17, 111.86, 102.50, 56.02, 39.32, 31.89, 31.68, 29.78, 26.74, 22.72, 14.55, 14.04; ESI-MS calculated for ($C_{25}H_{30}ClN_2O_3$) $[M + H]^+$: 441.19, found: 441.30.

*4.1.1.13. N,N′-[1,4-Phenylenebis(methylene)]bis-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide] (**13**).* It was prepared according to the method 2 except that 0.14 mmol *p*-xylylenediamine was used; white solid, yield 63.9%; ^{1}H NMR ($CDCl_3$) δ 8.59 (2H, br), 7.66 (7H, m), 7.15 (6H, s), 6.95 (2H, d, $J = 8.8$ Hz), 6.70 (2H, d, $J = 7.6$ Hz), 4.24 (4H, d, $J = 5.2$ Hz), 3.72 (6H, s), 3.58 (4H, s), 2.23 (6H, s).

*4.1.1.14. N,N′-[1,5-Pentanediyl]bis-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide] (**14**).* It was prepared according to the method 2 except that 0.14 mmol 1,5-diaminopentane was used; white solid, yield 28%; ^{1}H NMR ($CDCl_3$) δ 7.66 (4H, m), 7.48 (4H, m), 6.85 (4H, m), 6.67 (2H, dd, $J = 9.2$, 2.4 Hz), 5.69 (2H, br), 3.79 (6H, s), 3.61 (4H, s), 3.13 (4H, m), 2.38 (6H, s), 1.37 (4H, m), 1.62 (2H, m); ^{13}C NMR (DMSO-d_6) δ 170.23, 168.60, 156.18, 138.27, 135.81, 134.82, 131.77, 131.49, 130.90, 129.70, 115.23, 115.01, 111.87, 102.43, 56.02, 31.80, 29.30, 24.30, 14.00; ESI-MS calculated for ($C_{43}H_{43}Cl_2N_4O_6$) $[M + H]^+$: 781.26, found: 781.46.

*4.1.2. General procedure for preparation of Indomethacin derivatives (**15**–**24**)*

The coupling reaction between 5-methoxy-2-methyl-3-indoleacetic acid and the corresponding amine was carried out according to the procedure of method 2. The resulting product was used directly for the next step. To this intermediate (0.35 mmol) in anhydrous dimethylformamide was added sodium hydride (0.77 mmol, 18.5 mg) at 0–4 °C under argon and the mixture was stirred for 10 min. Then the substituted benzoyl chloride or benzenesulfonyl chloride (0.4 mmol) was added and stirred for half an hour. Then reaction solution was quenched with 20 ml aqueous sodium bicarbonate and extracted with ethyl acetate (3 × 20 ml). The organic layer was collected and concentrated under reduced pressure. The obtained product was purified by silica gel column chromatography.

*4.1.2.1. N-(2-Dimethylaminoethyl)-1-(phenylsulfonyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**15**).* 2-(Dimethylamino)ethylamine and benzenesulfonyl chloride were used; white solid, yield 30%; retention time: 3.5 min, purity: 99%; IR $\nu = 3305$ (N–H), 1648 (C=O); ^{1}H NMR ($CDCl_3$) δ 8.10 (1H, d, $J = 9.2$ Hz), 7.76 (2H, m), 7.55 (1H, m), 7.44 (2H, m), 6.91 (1H, dd, $J = 9.2$, 2.8 Hz), 6.84 (1H, d, $J = 2.4$ Hz), 6.02 (1H, br), 3.82 (3H, s), 3.53 (2H, s), 3.20 (2H, m), 2.55 (3H, s), 2.23 (2H, t, $J = 6$ Hz), 2.04 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.80, 156.95, 139.19, 135.66, 133.93, 130.99, 130.93, 129.53, 126.47, 115.74, 114.30, 113.35, 101.21, 57.83, 55.88, 45.14, 37.11, 32.46, 13.12; ESI-MS calculated for ($C_{22}H_{28}N_3O_4S$) $[M + H]^+$: 430.18, found: 430.29.

*4.1.2.2. N-(2-Dimethylaminoethyl)-1-(4-bromobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**16**).* 2-(Dimethylamino)ethylamine and 4-bromobenzoyl chloride were used; white solid, yield 14.3%; retention time: 4.4 min, purity: 99%; IR $\nu = 3292$ (N–H), 1637 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.61 (4H, m), 6.89 (2H, m), 6.70 (1H, m), 6.28 (1H, br), 3.83 (3H, s), 3.62 (2H, s), 3.28 (2H, m), 2.38 (3H, s), 2.32 (2H, t, $J = 6$ Hz), 2.10 (6H, s); ^{13}C NMR ($CDCl_3$) δ 170.16, 168.62, 156.37, 136.28, 134.39, 132.35, 131.47, 131.08, 130.64, 128.20, 115.27, 113.39, 112.32, 101.12, 57.89, 55.93, 45.26, 37.15, 32.40, 13.51; ESI-MS calculated for ($C_{23}H_{27}BrN_3O_3$) $[M + H]^+$: 472.12, found: 472.25.

*4.1.2.3. N-(2-Dimethylaminoethyl)-1-(4-methoxybenzoyl)-5-methoxy–2-methyl-1H-indole-3-acetamide (**17**).* 2-(Dimethylamino)ethylamine and 4-methoxybenzoyl chloride were used; white solid, yield 15.1%; ^{1}H NMR ($CDCl_3$) δ 7.70 (2H, m), 6.97 (2H, m), 6.91 (2H, m), 6.67 (1H, dd, $J = 9.2$, 2.8 Hz), 6.39 (1H, br), 3.91 (3H, s), 3.83 (3H, s), 3.64 (2H, s), 3.30 (2H, m), 2.38 (2H, t, $J = 6$ Hz), 2.39 (3H, s), 2.15 (6H, s); ^{13}C NMR (DMSO-d_6) δ 170.22, 168.98, 163.77, 155.85, 135.92, 132.65, 131.18, 127.78, 114.87, 114.71, 114.11, 111.72, 102.33, 58.91, 56.28, 56.07, 45.85, 37.56, 31.85, 13.68; ESI-MS calculated for ($C_{24}H_{30}N_3O_4$) $[M + H]^+$: 424.22, found: 424.33.

*4.1.2.4. N-(2-Dimethylaminoethyl)-1-(3,4-dimethoxybenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**18**).* 2-(Dimethylamino) ethylamine and 3,4-dimethoxybenzoyl chloride were used; white solid, yield 31%; ^{1}H NMR ($CDCl_3$) δ 7.36 (1H, d, $J = 2$ Hz), 7.30 (1H, dd, $J = 2$, 8.4 Hz), 6.91 (3H, m), 6.68 (1H, dd, $J = 8.8$, 2.4 Hz), 6.32 (1H, br), 3.98 (3H, s), 3.91 (3H, s), 3.83 (3H, s), 3.65 (2H, s), 3.29 (2H, m), 2.42 (3H, s), 2.32 (2H, t, $J = 6$ Hz), 2.10 (6H, s); ^{13}C NMR (DMSO-d_6) δ 170.17, 169.02, 155.86, 153.54, 149.33, 135.96, 131.24, 131.15, 127.72, 124.69, 114.75, 114.20, 112.93, 111.76, 102.29, 58.96, 56.46, 56.33, 56.05, 45.88, 37.59, 31.85, 13.69; ESI-MS calculated for ($C_{25}H_{32}N_3O_5$) $[M + H]^+$: 454.23, found: 454.35.

*4.1.2.5. N-(2-Dimethylaminoethyl)-1-(3,4,5-trimethoxybenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**19**).* 2-(Dimethylamino)ethylamine and 3,4,5-trimethoxybenzoyl chloride were used; white solid, yield 29%; retention time: 4.3 min, purity: 98%; IR $\nu = 1669$ (C=O); ^{1}H NMR ($CDCl_3$) δ 6.99 (2H, s), 6.92 (1H, d, $J = 2.4$ Hz), 6.89 (1H, d, $J = 9.2$ Hz), 6.68 (1H, dd, $J = 9.2$, 2.4 Hz), 6.29 (1H, br), 3.96 (3H, s), 3.83 (9H, s), 3.64 (2H, s), 3.29 (2H, m), 2.44 (3H, s), 2.32 (2H,t, $J = 6$ Hz), 2.11 (6H, s); ^{13}C NMR ($CDCl_3$) δ 170.48, 169.12, 156.16, 153.46, 142.47, 136.67, 131.25, 130.58, 130.29, 115.24, 113.10, 111.99, 107.56, 101.00, 61.33, 57.92, 56.62, 55.90, 45.01, 36.96, 32.41, 13.34; ESI-MS calculated for ($C_{26}H_{34}N_3O_6$) $[M + H]^+$: 484.24, found: 484.38.

*4.1.2.6. N-(2-Dimethylaminoethyl)-1-(2-naphthoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**20**).* 2-(Dimethylamino)ethylamine and 2-naphthoyl chloride were used; white solid, yield 30.7%; ^{1}H NMR ($CDCl_3$) δ 8.26 (1H, s), 7.93 (3H, m), 7.74 (1H, dd, J = 8.4, 1.6 Hz), 7.63 (2H, m), 6.95 (1H, d, J = 2.4 Hz), 6.87 (1H, d, J = 8.8 Hz), 6.61 (1H, dd, J = 8.8, 2.4 Hz), 6.39 (1H, br) 3.818 (3H, s), 3.66 (2H, s), 3.31 (2H, m), 2.41 (3H, s), 2.35 (2H, t, J = 6 Hz), 2.13 (6H, s); ^{13}C NMR (DMSO-d_6) δ 170.10, 169.64, 156.11, 135.98, 135.36, 133.40, 132.69, 131.52, 131.12, 131.02, 129.87, 129.32, 128.55, 127.90, 125.88, 115.06, 114.91, 111.83, 102.57, 58.81, 56.06, 45.70, 37.43, 31.86, 14.00; ESI-MS calculated for ($C_{27}H_{30}N_3O_3$) $[M + H]^+$: 444.23, found: 444.34.

*4.1.2.7. N-(2-(Pyrrolidin-1-yl)ethyl)-1-(3,4,5-trimethoxybenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**21**).* 1-(2-Aminoethyl) pyrrolidine and 3,4,5-trimethoxybenzoyl chloride were used; white solid, yield 15.4%; ^{1}H NMR ($CDCl_3$) δ 6.99 (2H, s), 6.92 (1H, d, J = 2.4 Hz), 6.89 (1H, d, J = 8.8 Hz), 6.68 (1H, dd, J = 2.4, 9.2 Hz), 6.53 (1H, br), 3.96 (3H, s), 3.83 (9H, s), 3.65 (2H, s), 3.34 (2H, m), 2.57 (2H, t, J = 6.4 Hz), 2.44 (7H, m), 1.66 (4H, m); ^{13}C NMR (DMSO-d_6) δ 170.14, 169.02, 156.10, 153.59, 141.86, 135.98, 131.41, 131.15, 131.10, 115.08, 114.81, 111.83, 107.55, 102.33, 61.03, 56.84, 56.05, 55.56, 54.27, 38.81, 31.83, 23.74, 13.92; ESI-MS calculated for ($C_{28}H_{36}N_3O_6$) $[M + H]^+$: 510.26, found: 510.40.

*4.1.2.8. N-(2-Morpholinoethyl)-1-(3,4,5-trimethoxybenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**22**).* 4-(2-Aminoethyl) morpholine and 3,4,5-trimethoxybenzoyl chloride were used; white product, yield 27.3%; ^{1}H NMR ($CDCl_3$) δ 6.99 (2H, s), 6.89 (2H, m), 6.70 (1H, dd, J = 8.8, 2.4 Hz), 6.33 (1H, br), 3.97 (3H, s) 3.83 (6H, s), 3.82 (3H, s), 3.66 (2H, s), 3.38 (4H, br), 3.29 (2H, m), 2.46 (3H, s), 2.36 (2H, t, J = 6 Hz), 2.24 (4H, m); ^{13}C NMR (DMSO-d_6) δ 170.23, 169.04, 156.10, 153.59, 141.88, 136.06, 131.38, 131.11, 131.12, 115.10, 114.70, 111.76, 107.54, 102.38, 66.73, 61.02, 57.96, 56.83, 56.06, 53.86, 36.64, 31.86, 13.91; ESI-MS calculated for ($C_{28}H_{36}N_3O_7$) $[M + H]^+$: 526.26, found: 526.40.

*4.1.2.9. N-(2-Morpholinoethyl)-1-(3,4-dimethoxybenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**23**).* 4-(2-Aminoethyl)morpholine and 3,4-dimethoxybenzoyl chloride were used; white solid, yield 17.1%; ^{1}H NMR ($CDCl_3$) δ 7.37 (1H, d, J = 2 Hz), 7.30 (1H, dd, J = 1.6, 8.4 Hz), 6.89 (3H, m), 6.68 (1H, dd, J = 9.2, 2.4 Hz), 6.34 (1H, br), 3.98 (3H, s), 3.92 (3H, s), 3.81 (3H, s), 3.67 (2H, s), 3.37 (4H, br), 3.29 (2H, m), 2.44 (3H, s), 2.35 (2H, t, J = 4 Hz), 2.24 (4H, m). ESI-MS calculated for ($C_{27}H_{34}N_3O_6$) $[M + H]^+$: 496.24, found: 496.38.

*4.1.2.10. N-(2-Morpholinoethyl)-1-(4-bromobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetamide (**24**).* 4-(2-Aminoethyl)morpholine and 4-bromobenzoyl chloride were used; white solid, yield 5.1%; ^{1}H NMR ($CDCl_3$) δ 7.63 (4H, m), 6.85 (2H, m), 6.69 (1H, d, J = 8.4 Hz), 6.30 (1H, br), 3.81 (3H, s), 3.65 (2H, s), 3.37 (4H, br), 3.29 (2H, m), 2.42 (3H, s), 2.36 (2H, t, J = 6 Hz), 2.24 (4H, br). ESI-MS calculated for ($C_{25}H_{29}BrN_3O_4$) $[M + H]^+$: 514.13, found: 514.28.

*4.1.3. General procedure for preparation of Sulindac derivatives (**25**–**35**)*

Sulindac derivatives **25**–**35** were prepared according to the procedure of method 2 with exception of using Sulindac instead of Indomethacin as starting material.

*4.1.3.1. N-(4-Methoxyphenyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**25**).* 4-Methoxyaniline was used; yellow solid, yield 69%; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, d, J = 8 Hz), 7.70 (2H, d, J = 8 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.21 (3H, m), 6.93 (1H, dd, J = 2.4, 8.4 Hz), 6.83 (2H, d, J = 8.8 Hz), 6.62 (1H, m), 3.77 (3H, s), 3.68 (2H, s), 2.83 (3H, s), 2.28 (3H, s); ^{13}C NMR ($CDCl_3$) δ 167.56, 163.68 (d, J = 239 Hz), 156.85, 146.58, 145.79, 141.68, 139.63, 139.19, 132.55, 130.70, 130.46, 129.76,129.09, 124.10,122.46, 114.28, 111.51 (d, J = 23 Hz), 106.32 (d, J = 24), 55.68, 44.10, 34.71, 10.92; ESI-MS calculated for $C_{27}H_{25}FNO_3S$ $[M + H]^+$: 462.15, found: 462.26.

*4.1.3.2. N-(4-Chlorophenyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**26**).* 4-Chloroaniline was used; yellow solid, yield 49%; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, d, J = 8 Hz), 7.70 (2H, d, J = 8 Hz), 7.39 (2H, d, J = 8.8 Hz), 7.34 (1H, br), 7.24 (4H, m), 6.91 (1H, dd, J = 2.4, 8.8 Hz), 6.62 (1H, m), 3.69 (2H, s), 2.83 (3H, s), 2.28 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.67, 163.21 (d, J = 242 Hz), 147.91 (d, J = 9.1 Hz), 146.92, 141.06, 139.20, 138.92, 138.69, 133.82, 130.61, 130.23, 130.09, 129.35, 127.56, 124.61, 123.79, 121.39, 111.09 (d, J = 22.1 Hz), 106.86 (d, J = 23.6 Hz), 43.78, 34.17, 11.16; ESI-MS calculated for $C_{26}H_{22}ClFNO_2S$ $[M + H]^+$: 466.10, found: 466.21.

*4.1.3.3. N-(4-Cyanophenyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**27**).* 4-Aminobenzonitrile was used; yellow solid, yield 22%; ^{1}H NMR ($CDCl_3$) δ 7.76 (2H, d, J = 8 Hz), 7.70 (2H, d, J = 8 Hz), 7.59 (4H, s), 7.49 (1H, br), 7.24 (2H, dd, J = 5.6, 8.4 Hz), 6.90 (1H, dd, J = 2.4, 8.4 Hz), 6.64 (1H, m), 3.72 (2H, s), 2.83 (3H, s), 2.29 (3H, s); ^{13}C NMR (DMSO-d_6) δ 169.40, 163.22 (d, J = 241.8 Hz), 147.85 (d, J = 8.4 Hz), 146.95, 143.92, 141.02, 139.17, 139.10, 134.02, 133.53, 130.61, 130.38, 130.09, 124.62, 123.83, 119.84, 119.71, 111.13 (d, J = 22.1 Hz), 106.83 (d, J = 23.6 Hz), 105.74, 43.77, 34.21, 11.16; ESI-MS calculated for $C_{27}H_{22}FN_2O_2S$ $[M + H]^+$: 457.14, found: 457.23.

*4.1.3.4. N-[2-(1-Pyrrolidinyl)ethyl]-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**28**).* 1-(2-Aminoethyl)pyrrolidine was used; yellow solid, yield 65%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8.0 Hz), 7.67 (2H, d, J = 8.0 Hz), 7.18 (2H, m), 6.88 (1H, dd, J = 2.8, 8.8 Hz), 6.58 (1H, m), 6.43 (1H, br), 3.52 (2H, s), 3.31 (2H, m), 2.82 (3H, s), 2.53 (2H, t, J = 6 Hz), 2.39 (4H, m), 2.21 (3H, s), 1.64 (4H, m); ^{13}C NMR ($CDCl_3$) δ 169.24, 162.56 (d, J = 250 Hz), 146.78, 145.75, 141.83, 139.77, 138.59, 133.16, 130.41, 129.75, 128.47, 124.08, 123.91 (d, J = 9 Hz), 111.24 (d, J = 22), 106.32 (d, J = 24 Hz), 54.12, 53.82, 44.12, 38.27, 33.96, 23.65, 10.75; ^{13}C NMR ($CDCl_3$) δ 169.24, 163.56 (d, J = 250.5 Hz), 146.78, 145.75, 141.83, 139.77, 138.59, 133.16, 130.41, 129.75, 128.47, 124.08, 123.91 (d, J = 8.7 Hz), 111.23 (d, J = 22.5 Hz), 106.31 (d, J = 23.7 Hz), 54.12, 53.82, 44.12, 38.27, 33.96, 23.65, 10.75; ESI-MS calculated for $C_{26}H_{30}FN_2O_2S$ $[M + H]^+$: 453.20, found: 453.30.

*4.1.3.5. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**29**).* 2-(Dimethylamino)ethylamine was used; yellow solid, yield 80%; ^{1}H NMR ($CDCl_3$) δ 7.73 (2H, d, J = 8.8 Hz), 7.67 (2H, d, J = 8.8 Hz), 7.18 (2H, m), 6.88 (1H, d, J = 2.4, 8.8 Hz), 6.58 (1H, m), 6.28 (1H, br), 3.51 (2H, s), 3.29 (2H, q, J = 5.6 Hz), 2.82 (3H, s), 2.34 (2H, t, J = 5.6 Hz), 2.216 (3H, s), 2.14 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.35, 163. 55 (d, J = 245 Hz), 146.82 (d, J = 8.7 Hz), 145.68, 141.83, 139.79, 138.57, 133.09, 130.45, 129.80, 128.47, 124.04, 123.88 (d, J = 8 Hz), 111.13 (d, J = 23 Hz), 106.35 (d, J = 24 Hz), 57.86, 45.24, 44.11, 37.23, 33.97, 10.78; ESI-MS calculated for $C_{24}H_{28}FN_2O_2S$ $[M + H]^+$: 427.19, found: 427.28.

*4.1.3.6. N-[3-(Dimethylamino)propyl]-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**30**).* 3-(Dimethylamino)propylamine was used; yellow solid, yield 57%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, m), 7.66 (3H, m), 7.19 (2H, m), 6.86 (1H, dd, J = 2.4, 8.8 Hz), 6.58 (1H, m), 3.50 (2H, s), 3.35 (2H, q, J = 5.6 Hz), 2.82 (3H, s), 2.24 (2H, t, J = 5.6 Hz), 2.20 (3H, s), 1.88

(6H, s), 1.54 (2H, m); ^{13}C NMR ($CDCl_3$) δ 169.32, 163.66 (d, J = 246 Hz), 147.08, 145.81, 141.99, 139.69, 138.67, 133.13, 130.34, 129.81, 128.33, 124.12, 123.86 (d, J = 9 Hz), 111.21 (d, J = 23 Hz), 106.30 (d, J = 24 Hz), 59.50, 45.14, 44.12, 40.77, 34.02, 25.06, 10.70; ESI-MS calculated for $C_{25}H_{30}FN_2O_2S$ $[M + H]^+$: 441.20, found: 441.30.

*4.1.3.7. N-(n-Butyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**31**).* n-Butylamine was used; yellow solid, yield 84%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.4 Hz), 7.20 (2H, m), 6.86 (1H, dd, J = 2.4, 8.8 Hz), 6.60 (1H, dt, J = 2, 8.8 Hz), 5.60 (1H, br), 3.52 (2H, s), 3.22 (2H, q, J = 6.8 Hz), 2.82 (3H, s), 2.21 (3H, s), 1.41 (2H, m), 1.25 (2H, m), 0.87 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 169.22, 163.58 (d, J = 246 Hz), 146.64 (d, J = 9 Hz), 145.71, 141.69, 139.67, 138.84, 132.91, 130.45, 129.72, 128.81, 124.07, 123.95, 111.33 (d, J = 22 Hz), 106.29 (d, J = 24 Hz), 44.07, 39.68, 33.95, 31.77, 20.18, 13.91, 10.79; ESI-MS calculated for $C_{24}H_{27}FNO_2S$ $[M + H]^+$: 412.17, found: 412.26.

*4.1.3.8. N-(n-Hexyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**32**).* n-Hexylamine was used; yellow solid, yield 76%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8.4 Hz), 7.68 (2H, d, J = 8.4 Hz), 7.20 (2H, m), 6.86 (1H, dd, J = 2.4, 8.8 Hz), 6.60 (1H, m), 5.58 (1H, br), 3.52 (2H, s), 3.21 (2H, q, J = 6.8 Hz), 2.82 (3H, s), 2.21 (3H, s), 1.42 (2H, m), 1.21 (6H, m), 0.84 (3H, t, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 169.11, 163.61 (d, J = 246 Hz), 146.61 (d, J = 9 Hz), 145.80, 141.69, 139.64, 138.84, 132.92, 130.44, 129.71, 128.87, 124.08, 123.98, 111.41 (d, J = 23 Hz), 106.31 (d, J = 24 Hz), 44.10, 39.93, 34.01, 31.58, 29.65, 26.65, 22.72, 14.18, 10.79; ESI-MS calculated for $C_{26}H_{31}FNO_2S$ $[M + H]^+$: 440.21, found: 440.30.

*4.1.3.9. N-(n-Octyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**33**).* n-Octylamine was used; yellow solid, yield 76%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.4 Hz), 7.20 (2H, m), 6.86 (1H, dd, J = 2.4, 8.8 Hz), 6.60 (1H, m), 5.59 (1H, br), 3.52 (2H, s), 3.21 (2H, q, J = 7.2 Hz), 2.82 (3H, s), 2.21 (3H, s), 1.42 (2H, m), 1.21 (10H, m), 0.86 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 169.16, 163.61 (d, J = 246 Hz), 146.64 (d, J = 8.4 Hz), 145.78, 141.72, 139.67, 138.84, 132.92, 130.45, 129.73, 129.76, 128.85, 124.08, 123.98, 111.39 (d, J = 22.9 Hz), 106.34 (d, J = 23.6 Hz), 44.09, 39.94, 34.00, 31.93, 29.70, 29.39, 27.02, 22.83, 14.30, 10.79; ESI-MS calculated for $C_{28}H_{35}FNO_2S$ $[M + H]^+$: 468.24, found: 468.34.

*4.1.3.10. N-(1-Methylhexyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**34**).* 2-Aminoheptane was used; yellow solid, yield 61%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8 Hz), 7.69 (2H, d, J = 8 Hz), 7.20 (2H, m), 6.86 (1H, dd, J = 2.4, 8.8 Hz), 6.60 (1H, m), 5.30 (1H, d, J = 8.4 Hz), 3.91 (1H, m), 3.51 (2H, s), 2.82 (3H, s), 2.21 (3H, s), 1.34–1.17 (8H, m), 1.06 (3H, d, J = 6.4 Hz), 0.83 (3H, t, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 168.46, 163.61 (d, J = 246 Hz), 146.69, 145.79, 141.73, 139.68, 138.77, 133.01, 130.47, 129.72, 128.80, 124.08, 123.98, 111.38 (d, J = 22.9 Hz), 106.30 (d, J = 23.7 Hz), 45.71, 44.11, 36.83, 34.20, 31.75, 25.77, 22.71, 21.14, 14.18, 10.78; ESI-MS calculated for $C_{27}H_{33}FNO_2S$ $[M + H]^+$: 454.22, found: 454.32.

*4.1.3.11. N-(3-Methylbutyl)-(Z)-5-fluoro-2-methyl-1-{[4-(methylsulfinyl)phenyl]methylene}-1H-indene-3-acetamide (**35**).* 3-Methylbutylamine was used; yellow solid, yield 66%; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.4 Hz), 7.20 (2H, m), 6.85 (1H, dd, J = 2.4, 8.8 Hz), 6.61 (1H, m), 5.53 (1H, br), 3.52 (2H, s), 3.24 (2H, m), 2.82 (3H, s), 2.21 (3H, s), 1.50 (1H, m), 1.30 (2H, m), 0.86 (6H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 169.07, 163.62 (d, J = 246.4 Hz), 146.57, 145.85, 141.71, 139.65, 138.85, 132.89, 130.45, 129.73, 128.86, 124.09, 123.99, 111.41 (d, J = 22.2 Hz), 106.32 (d, J = 23.6 Hz), 44.12, 38.58, 38.31, 34.02, 26.10, 22.62, 10.79; ESI-MS calculated for $C_{25}H_{29}FNO_2S$ $[M + H]^+$: 426.19, found: 426.28.

*4.1.4. General procedure for preparation of Sulindac derivatives (**36**–**41**)*

The coupling reaction between 2-(5-fluoro-2-methyl-1*H*-inden-3-yl)acetic acid and 2-dimethylaminoethylamine was carried out according to the procedure of method 2. The resulting product was used directly for the next step. To a solution of *N*-(2-(dimethylamino) ethyl)-2-(5-fluoro-2-methyl-1*H*-inden-3-yl)acetamide (110 mg, 0.4 mmol) and the appropriate aldehyde (0.44 mmol) in 2 ml methanol was added 0.5 ml of 1 N aqueous sodium hydroxide. The mixture was stirred at reflux for 2 h. The solution was cooled and then diluted with water. The precipitated solid was filtered and washed with water. Finally, the crude product was subjected to recrystallization or column chromatography to afford the final product.

*4.1.4.1. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-{[4-(methylthio)phenyl]methylene}-1H-indene-3-acetamide (**36**).* 4-Methylthiobenzyldehyde was used; yellow solid, yield 37%; ^{1}H NMR ($CDCl_3$) δ 7.45 (2H, m), 7.39 (1H, dd, J = 5.2, 8.4 Hz), 7.29 (2H, m), 7.16 (1H, s), 6.87 (1H, dd, J = 2.4, 8.8 Hz), 6.60 (1H, m), 6.23 (1H, br), 3.51 (2H, s), 3.28 (2H, m), 2.55 (3H, s), 2.32 (2H, t, J = 6.4 Hz), 2.21 (3H, s), 2.11 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.56, 163.33 (d, J = 244.9 Hz), 146.52 (d, J = 9.1 Hz), 140.23, 139.51, 138.91, 133.02, 131.94, 130.25, 130.12, 126.09, 123.93 (d, J = 8.4 Hz), 110.96 (d, J = 22.1 Hz), 105.98 (d, J = 23.6 Hz), 57.88, 45.26, 37.26, 34.00, 15.58, 10.81; ESI-MS calculated for $C_{24}H_{28}FN_2OS$ $[M + H]^+$: 411.19, found: 411.29.

*4.1.4.2. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-[(4-methoxyphenyl)methylene]-1H-indene-3-acetamide (**37**).* 4-Methoxybenzaldehyde was used; yellow solid, yield 37%; ^{1}H NMR ($CDCl_3$) δ 7.48 (2H, m), 7.43 (1H, dd, J = 5.2, 8.4 Hz), 7.19 (1H, s), 6.97 (2H, m), 6.87 (1H, dd, J = 2.4, 8.8 Hz), 6.61 (1H, m), 6.23 (1H, br), 3.89 (3H, s), 3.52 (2H, s), 3.28 (2H, m), 2.31 (2H, t, J = 6 Hz), 2.21 (3H, s), 2.11 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.65, 163.23 (d, J = 244.1 Hz), 159.96, 146.37, 139.46, 139.05, 131.46, 131.19, 130.85, 130.21, 128.83, 123.78 (d, J = 8.4 Hz), 114.16, 110.87 (d, J = 22.9 Hz), 105.85 (d, J = 23.6 Hz), 57.88, 55.58, 45.26, 37.26, 34.01, 10.82; ESI-MS calculated for $C_{24}H_{28}FN_2O_2$ $[M + H]^+$: 395.21, found: 395.30.

*4.1.4.3. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-[(3,4,5-trimethoxyphenyl)methylene]-1H-indene-3-acetamide (**38**).* 3,4,5-Trimethoxybenzaldehyde was used; yellow solid, yield 50%; ^{1}H NMR ($CDCl_3$) δ 7.46 (1H, dd, J = 5.6, 8.4 Hz), 7.17 (1H, s), 6.89 (1H, dd, J = 2.4, 8.8 Hz), 6.76 (2H, s), 6.62 (1H, m), 6.280 (1H, br), 3.93 (3H, s), 3.85 (6H, s), 3.52 (2H, s), 3.29 (2H, m), 2.34 (2H, t, J = 6 Hz), 2.21 (3H, s), 2.13 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.62, 163.36 (d, J = 245 Hz), 153.40, 146.61, 140.30, 138.83, 138.28, 132.01, 131.95, 130.64, 130.02, 124.23 (d, J = 9.2 Hz), 110.87 (d, J = 22.1 Hz), 106.67, 106.06 (d, J = 23.7 Hz), 61.29, 57.97, 56.40, 45.27, 37.27, 33.94, 10.79; ESI-MS calculated for $C_{26}H_{32}FN_2O_4$ $[M + H]^+$: 455.23, found: 455.33.

*4.1.4.4. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-[(4-chlorophenyl)methylene]-1H-indene-3-acetamide (**39**).* 4-Chlorobenzaldehyde was used; yellow solid, yield 31%; ^{1}H NMR ($CDCl_3$) δ 7.42 (4H, m), 7.23 (1H, dd, J = 5.2, 8.4 Hz), 7.14 (1H, s), 6.87 (1H, dd, J = 2.4, 8.8 Hz), 6.59 (1H, m), 6.24 (1H, br), 3.51 (2H, s), 3.28 (2H, m), 2.32 (2H, t, J = 6 Hz), 2.20 (3H, s), 2.12 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.41, 163.46 (d, J = 245.7 Hz), 146.71, 141.09, 138.68, 135.11, 134.38, 132.57, 130.86, 129.95, 129.03, 123.92 (d, J = 9.1 Hz), 111.08 (d, J = 22.9 Hz), 106.19 (d, J = 23.6 Hz), 57.85, 45.26, 37.25, 34.00, 10.77; ESI-MS calculated for $C_{23}H_{25}ClFN_2O$ $[M + H]^+$: 399.16, found: 399.26.

*4.1.4.5. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-[(4-bromophenyl)methylene]-1H-indene-3-acetamide (**40**).* 4-Bromob enzaldehyde was used; yellow solid, yield 50%; ^{1}H NMR ($CDCl_3$) δ 7.57 (2H, m), 7.38 (2H, m), 7.23 (1H, dd, $J = 5.2, 8.4$), 7.11 (1H, s), 6.87 (1H, dd, $J = 2.4, 8.8$ Hz), 6.59 (1H, m), 6.24 (1H, br), 3.50 (2H, s), 3.28 (2H, m), 2.32 (2H, t, $J = 6$ Hz), 2.20 (3H, s), 2.12 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.39, 163.46 (d, $J = 245.7$ Hz), 146.68 (d, $J = 8.4$ Hz), 141.09, 138.67, 135.58, 132.63, 131.96, 131.14, 129.91, 129.02, 123.93 (d, $J = 9.2$ Hz), 122.59, 111.08 (d, $J = 22.9$ Hz), 106.20 (d, $J = 23.7$ Hz), 57.84, 45.26, 37.26, 33.99, 10.76; ESI-MS calculated for $C_{23}H_{25}BrFN_2O$ $[M + H]^+$: 443.11, found: 443.21.

*4.1.4.6. N-[2-(Dimethylamino)ethyl]-(Z)-5-fluoro-2-methyl-1-[(2-naphthyl)methylene]-1H-indene-3-acetamide (**41**).* 2-Naphthalde hyde was used; yellow solid, yield 30%; ^{1}H NMR ($CDCl_3$) δ 7.99 (1H, s), 7.86 (3H, m), 7.62 (1H, dd, $J = 8.4, 1.6$ Hz), 7.55 (2H, m), 7.38 (1H, s), 7.31 (1H, dd, $J = 5.2, 8.4$ Hz), 6.89 (1H, dd, $J = 8.8, 2.4$ Hz), 6.54 (1H, m), 6.28 (1H, br), 3.54 (2H, s), 3.30 (2H, m), 2.33 (2H, t, $J = 6$ Hz), 2.26 (3H, s), 2.13 (6H, s); ^{13}C NMR ($CDCl_3$) δ 169.55, 163.42 (d, $J = 244.9$ Hz), 146.61 (d, $J = 9.2$ Hz), 140.77, 138.93, 134.11, 133.40, 133.21, 132.23, 132.25, 130.69, 130.17, 130.20, 128.90, 128.37, 128.35, 128.05, 127.31, 126.88, 126.79, 124.01 (d, $J = 8.4$ Hz), 111.03 (d, $J = 22.9$ Hz), 106.04 (d, $J = 23.6$ Hz), 57.89, 45.27, 37.28, 34.04, 31.16, 10.84; ESI-MS calculated for $C_{27}H_{28}FN_2O$ $[M + H]^+$: 415.22, found: 415.31.

4.2. Cell viability analysis

The effect of Indomethacin and Sulindac derivatives on cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay (MTT) in quadruplicates with HT29 colon cancer cells [39]. HT29 cells were maintained in RPMI 1640 medium supplemented with 10% heat activated fetal bovine serum (FBS), 2 mmol/l L-Glutamine, 1 mmol/l sodium pyruvate, 100 U/ml penicillin-streptomycin. The culture medium was changed every four days. Cell cultures were grown at 37 °C in a humidified atmosphere of 5% CO_2 in a Heraeus CO_2 incubator. 4000 cells were seeded per well with culture medium in 96-well, flat-bottomed plates and allowed to attach and grow for 24 h. The cells reached about 30%–35% confluency at this stage. The cells were then exposed to various concentrations (include 1000, 200, 40, 8, 1.6, and 0.32 μM, with a dilution factor of 5) of Indomethacin and Sulindac derivatives dissolved in DMSO (final concentration $\leq$ 0.1%) in media for 72 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells, and the control cells reached about 90%–95% confluency after the treatment. The morphology of the control cells will be monitored to ensure the health of the cells before the MTT assay. The medium was removed, replaced by 200 μl of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) in fresh media, and cells were incubated in the CO_2 incubator at 37 °C for 2 h. Supernatants were removed from the wells, and the reduced MTT was solubilized in 200 μl/well DMSO. Absorbance at 570 nm was determined on a plate reader.

4.3. Tubulin polymerization assay

200 μl microtubule-associated protein-rich tubulin (2 mg/ml, bovine brain, Cytoskeleton) in buffer containing 80 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 0.5 mM EGTA, and 5% glycerol was mixed with DMSO (as control) or various concentrations of Indomethacin derivatives in DMSO and incubated at 37 °C. 2 μl of 1 mM GTP was added to the mixture to initiate the tubulin polymerization and the absorbance at 340 nm was monitored over 20 min using a Varian Cary 50 series spectrophotometer.

4.4. Molecular docking simulation

Molecular docking of compound **19** into the crystal structure of tubulin was performed using software AUTODOCK 4 [40]. The X-ray crystal structures of tubulin with PDB ID: 1SA0 and 1JFF from Protein Data Bank were used for docking simulation of compound **19** into the colchicine binding site and taxoid binding site respectively. For preparing tubulin for autodock, all hydrogens were added and the ligands with identifier CN2700 in 1SA0 and TA1601 in 1JFF were deleted. Compound **19** were drawn and energy minimized with MM2 force field using Chem3D Ultra 10.0 (Cambridge Soft Corp., US).

Autogrid was used to pre-calculate the grid maps of the binding energy between tubulin and compound **19**. For compound **19** binding into the colchicine binding site, a grid box size of $44 \times 46 \times 42$ points in x, y and z directions was built and the grid center was located in $x = 116.909$, $y = 89.688$, $z = 7.094$. For compound **19** binding into the taxoid binding site, a grid box size of $40 \times 42 \times 40$ points in x, y and z directions was built and the grid center was located in $x = 1.403$, $y = -16.979$, $z = 16.391$. For running autodock, Lamarckian genetic algorithm was chosen as the search method. Genetic algorithm parameters were set as default. The 10 docked conformations were generated and the conformation with the lowest docked energy was selected as the most probable binding mode. The estimated lowest free energies for binding at the cochinchine site and taxoid site are −10.43 and −8.44 kcal/mol respectively.

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