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Original article

Regioselective synthesis of dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-diones of potential anti-tumor properties

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Abstract

1,3-Dipolar cycloaddition reaction of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones **1a–g** with non-stabilized azomethine ylides, generated in situ via decarboxylative condensation of isatins **2a**,**b** and sarcosine (**3**) afforded dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-diones **4a–n** and not the isomeric forms dispiro[1*H*-indene-2,4′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-diones **5** in a highly regioselective manner. Anti-tumor activity screening for the synthesized compounds (**4c**,**d**,**i–l**) at a dose of 10 μM utilizing 56 different human tumor cell lines representing, leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney was carried out. All the tested compounds exhibit promising anti-tumor activity against SK-MEL-2 (melanoma) cell line. Anti-inflammatory activity of the prepared compounds was determined in vivo by the acute carrageenan-induced paw oedema in rats. Many of the prepared compounds exhibit considerable anti-inflammatory properties ''at a dose of 50 mg/kg body weight'', especially **4a** and **4m** which reveal remarkable activities relative to indomethacin which was used as a reference standard in this study.

Keywords: 2-(Arylmethylene)-2,3-dihydro-1*H*-inden-1-ones; Azomethine ylides; Dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-dione; Anti-tumor; Anti-inflammatory

1. Introduction

Spiropyrrolidinyl-oxindole represents the main alkaloid skeleton of naturally occurring substances such as spirotryprostatine A and spirotryprostatine B [1,2] which were found to be inhibitors of mammalian cell cycle at G2/M phase, from the secondary metabolites of *Aspergillus fugimatus*. Elacomine [3] was isolated from *Eleagnus commutata* and horsfiline [4–9] was isolated from *Horsfieldia superba*, a small Malaysian tree, extracts of which have found use in indigenous medicine. Mitraphylline was isolated from *Uncaria tomentosa* (cat's claw) and identified as anti-tumor active agent against human brain cancer cell lines, neuroblastoma SKN-BE(2) and malignant glioma GAMG [10] (Scheme 1). Generally, several oxindole derivatives are well known as anti-tumor active agents due to their kinase inhibitory properties [11–15] especially, tyrosine kinase inhibitory effects [11–13].

In the present work, it is intended to utilize a natural product isatin scaffold for formation of non-stabilized azomethine ylides following the previously described and successful methods [16–19] through decarboxylative condensation with α-amino acids and trapping the generated reactive intermediates via 1,3-dipolar cycloaddition reaction with the exocyclic olefinic linkage derived from 1-indanone. Utilization of exocyclic olefinic linkage containing-compounds as dipolarophiles in such reactions should afford directly spiropyrrolidinyl-oxindole analogues having a skeleton of biologically active and naturally occurring spiro-alkaloids. Investigations on the regioselectivity of the reactions as well as stereochemical structure of the isolated products are the main chemical objective targets of this study. The anti-tumor as well as anti-inflammatory properties of the prepared compounds will be screened. This work is considered a continuation of our research activity directed towards construction of bio-active heterocycles,

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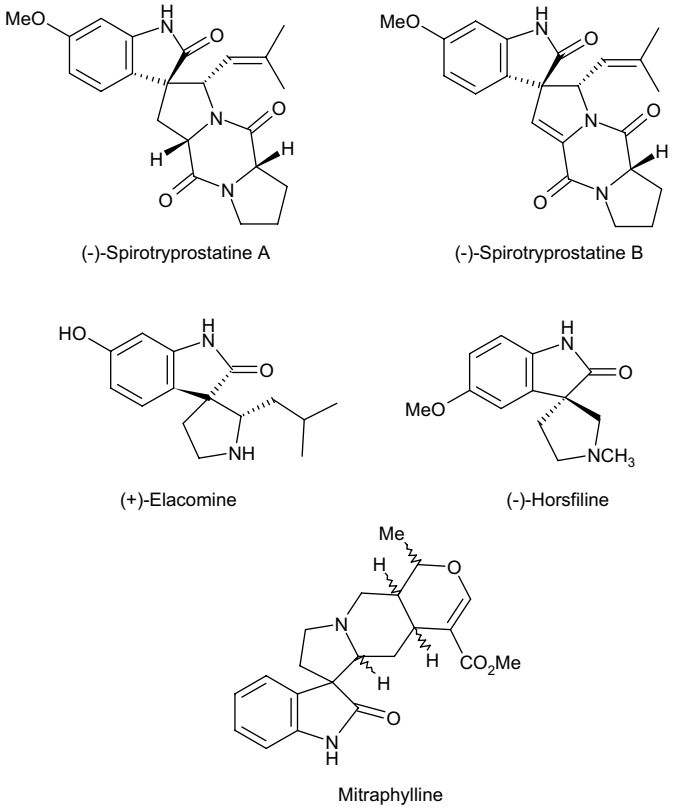


Scheme 1.

especially those associated with anti-tumor [20] and anti-inflammatory [21–24] properties.

The pharmacological activities of 1*H*-indene containing-compounds also prompted this study. For example, 2-aryl-1*H*-indene derivatives were reported to be effective COX-2 inhibitors, however, few analogues are selective COX-1 inhibitors [25]. Experimental and clinical results have suggested a possible involvement of COX-1 in cancer and pain development [26–29]. Other reports explained that 2-substituted-1-indanones are effective acetylcholinesterase inhibitors [30]. Acetylcholinesterase inhibitors are the first and the most developed group of drugs approved for Alzheimer's disease symptomatic treatment [30]. Additionally, other derivatives were reported as allosteric potentiators of the metabotropic glutamate subtype 2 receptor ($mGluR_2$) [31]. Agents targeting $mGluR_2$ may have utility in a variety of clinical conditions [32–34], including epilepsy, anxiety and schizophrenia [35].

2. Results and discussion

2.1. Chemistry

Reaction of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones **1a–g** with non-stabilized azomethine ylides, generated in situ via decarboxylative condensation of sarcosine (**3**) "as a representative example of α-amino acid" and isatins **2a,b** in refluxing ethanol afforded only one product as indicated by TLC in a highly regioselective manner (Scheme 2). The structure of the isolated product was established to be dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-dione **4** rather than the regioisomeric form dispiro[1*H*-indene-2,4′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-dione **5** based on spectroscopic (IR, ^{1}H, ^{13}C NMR, HMQC, MS) and elemental analyses data.

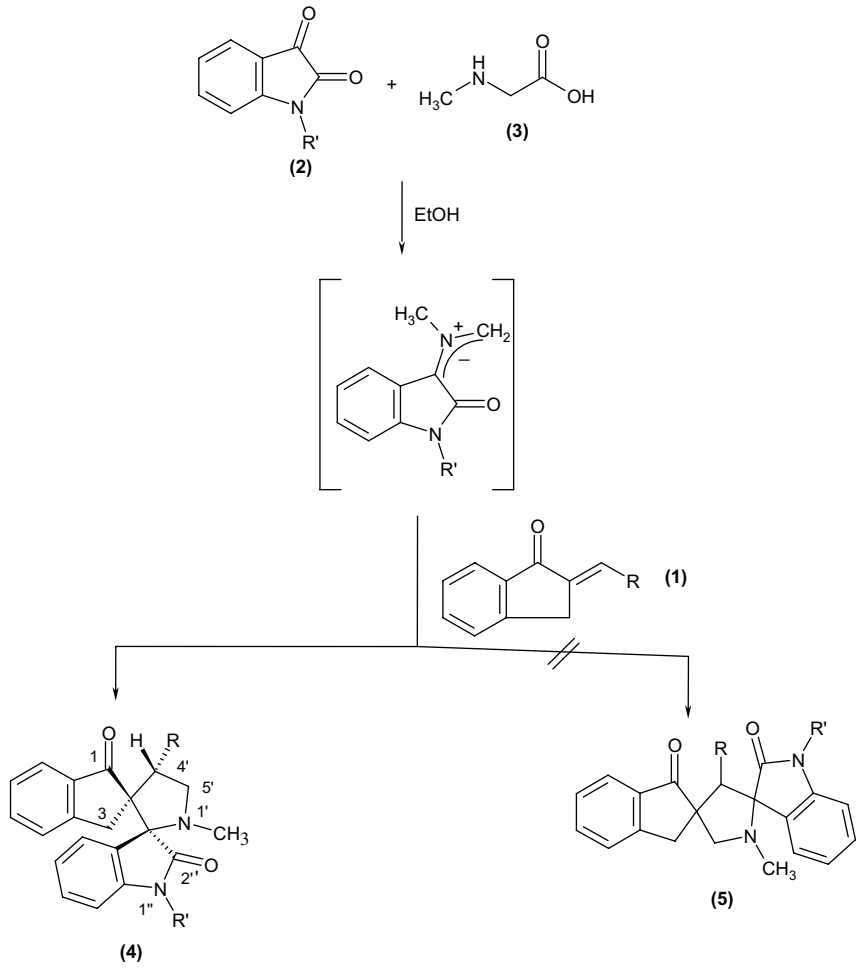
^{1}H NMR spectra of **4a–n** reveal the presence of three signal sets at $\delta = 3.61-3.74$, 4.04–4.13 (assignable for the non-magnetically equivalent pyrrolidinyl methylene protons H_2C-5′, coupled with each other and in turn with the vicinal methine proton *HC*-4′) and 4.31–4.62 (corresponding to the pyrrolidinyl methine proton *HC*-4′), excluding the formation of the other regioisomeric form **5**. In addition two doublet signals at $\delta = 2.67-2.87$, 2.87–3.13 ($J = 17.7-18.0$ Hz) corresponding to the indanyl methylene protons H_2C-3 were observed. ^{13}C NMR spectrum of **4b** "as a representative example" exhibits the pyrrolidinyl spiro-carbons *C*-3′ (*C*-2), *C*-2′ (*C*-3″) at $\delta = 65.88$, 77.30, besides the pyrrolidinyl methine carbon (*HC*-4′) at $\delta = 49.36$. The indanyl (H_2C-3) and pyrrolidinyl (H_2C-5′) methylene carbons appeared at $\delta = 34.48$, 58.37. In addition, the oxindolyl (*C*-2″) and indanyl (*C*-1) carbonyl carbons are recognized at $\delta = 175.53$, 205.44. The oxindolyl and pyrrolidinyl methyl carbons (NCH_3) are exhibited at $\delta = 25.26$, 34.39, respectively.

Heteronuclear multi-quantum coherence (HMQC) spectrum of **4b** "as a representative example" adds a good support for the established structure. It reveals that the pyrrolidinyl methylene carbon (*C*-5′, at $\delta = 58.37$) correlates with the two signal sets appearing at $\delta = 3.62$, 4.13. However, the pyrrolidinyl methine carbon (*C*-4′ at $\delta = 49.36$) only correlates with the double doublet signal appearing at $\delta = 4.36$. It has also been noticed that the methylene indanyl carbon (H_2C-3 at $\delta = 34.48$) correlates with two doublet signals appearing at $\delta = 2.70$, 2.90. These observations add sharp evidence for the established spectral interpretations. Mass spectra (EI) of **4a–n** reveal the (M + 1) signals in considerable relative intensity values consistent with the established structure. Single crystal X-ray diffraction of **4n** (Fig. 1) adds a conclusive support for the established structure and indicates that the isolated crystalline form product is 2*S*, 3″*R*, 4′*R*.

2.2. Anti-tumor activity

Anti-tumor activity screening for the synthesized compounds (**4c,d,i–l**) at a dose of 10 μM utilizing 56 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney was carried out according to the previously reported standard procedure [20,36–38]. The obtained results (Table 1) represent percentage growth of the tumor cell lines treated with compounds under investigation relative to control cell experiments.

From the observed data it has been noticed that the tested compounds reflect moderate or no activity at all against most of the used human tumor cells. However, few promising anti-tumor properties were exhibited against scattered tumor cell lines, considering that cell line growth inhibition with >50% at a concentration of 10 μM usually seems to be



1a; R = Ph
1b; R = 4-ClC_6H_4
1c; R = 4-FC_6H_4
1d; R = 4-$H_3CC_6H_4$
1e; R = 4-$H_3COC_6H_4$
1f; R = 2-thienyl
1g; R = 2-furanyl

2a; R' = H
2b; R' = CH_3

4a; R = Ph, R' = H
4b; R = Ph, R' = CH_3
4c; R = 4-ClC_6H_4, R' = H
4d; R = 4-ClC_6H_4, R' = CH_3
4e; R = 4-FC_6H_4, R' = H
4f; R = 4-FC_6H_4, R' = CH_3
4g; R = 4-$H_3CC_6H_4$, R' = H
4h; R = 4-$H_3CC_6H_4$, R' = CH_3
4i; R = 4-$H_3COC_6H_4$, R' = H
4j; R = 4-$H_3COC_6H_4$, R' = CH_3
4k; R = 2-thienyl, R' = H
4l; R = 2-thienyl, R' = CH_3
4m; R = 2-furanyl, R' = H
4n; R = 2-furanyl, R' = CH_3

Scheme 2.

a noticeable activity. It has also been noticed that all the tested compounds exhibit remarkable anti-tumor activity against SK-MEL-2 (melanoma). This may attract the attention towards selective tumor inhibitory properties of the constructed ring system. Structure–activity relationships considering these latter observations explain that attachment of methyl group at the 1″-position of the prepared compounds usually associates with enhancement in anti-tumor properties as indicated in pairs **4c**, **4d** (percentage growth of cell line −6.14, −11.05, respectively), **4i**, **4j** (percentage growth of cell line 8.70, −6.92, respectively) and **4k**, **4l** (percentage growth of cell line 6.58, −5.11, respectively). It has also been noticed that attachment of 4′-position of the constructed compounds with *p*-chlorophenyl function (deactivating residue attached to the phenyl group) seems more suitable for developing an anti-tumor active agent than the cases of either utilizing *p*-methoxyphenyl (electron-donating residue attached to the phenyl group) or thienyl (heterocyclic residue) functions, as exhibited in cases of **4c**, **4i**, **4l** (percentage growth of cell line −6.14, 8.70, 6.58, respectively) and **4d**, **4j**, **4l** (percentage growth of cell line −11.05, −6.92, −5.11, respectively).

2.3. Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds **4a**,**c**–**n** (at a dose of 50 mg/kg body weight) was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats [22–24,39]. The anti-inflammatory

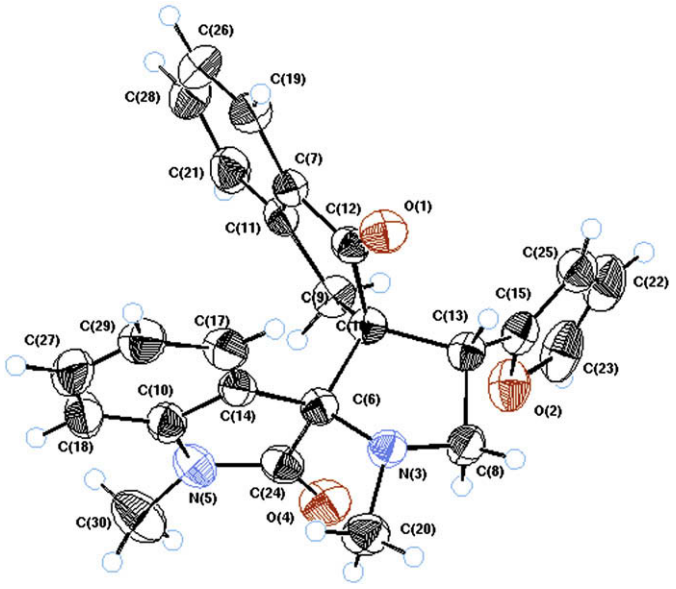


Fig. 1. ORTEP projection of single crystal X-ray diffraction of **4n**. Selected intramolecular bond lengths (Å) and bond angles (°) of **4n**. O(1)–C(12) = 1.225(2), O(2)–C(15) = 1.377(2), O(2)–C(23) = 1.378(2), N(3)–C(6) = 1.460(2), N(3)–C(8) = 1.453(2), N(3)–C(20) = 1.455(2), O(4)–C(24) = 1.220(2), N(5)–C(10) = 1.405(2), N(5)–C(24) = 1.361(2), N(5)–C(30) = 1.459(3), C(6)–C(14) = 1.516(2), C(6)–C(16) = 1.585, C(6)–C(24) = 1.556, C(7)–C(11) = 1.385(2), C(7)–C(12) = 1.459(2), C(7)–C(19) = 1.395(2), C(8)–C(13) = 1.528, C(9)–C(11) = 1.497(2), C(9)–C(16) = 1.552, C(10)–C(14) = 1.393(2), C(10)–C(18) = 1.382(3), C(11)–C(21) = 1.392(2), C(12)–C(16) = 1.543, C(13)–C(15) = 1.482(2), C(13)–C(16) = 1.574, C(14)–C(17) = 1.383(2), C(15)–C(25) = 1.344(3), C(17)–C(29) = 1.384(3), C(18)–C(27) = 1.376(3), C(19)–C(26) = 1.379(3), C(21)–C(28) = 1.385(3), C(22)–C(23) = 1.320(3), C(22)–C(25) = 1.424(3), C(26)–C(28) = 1.381(3), C(27)–C(29) = 1.373(3), C(15)–O(2)–C(23) = 106.0(2), C(6)–N(3)–C(8) = 106.97(12), C(6)–N(3)–C(20) = 115.20(13), C(8)–N(3)–C(20) = 114.53(14), C(10)–N(5)–C(24) = 111.06(15), C(10)–N(5)–C(30) = 124.8(2), C(24)–N(5)–C(30) = 123.6(2), N(3)–C(6)–C(14) = 113.60(13), N(3)–C(6)–C(16) = 102.59, N(3)–C(6)–C(24) = 113.19, C(14)–C(6)–C(16) = 116.88, C(11)–C(7)–C(12) = 109.21(15), C(11)–C(7)–C(19) = 121.9(2), C(12)–C(7)–C(19) = 128.8(2), N(3)–C(8)–C(13) = 102.70, C(11)–C(9)–C(16) = 105.53, N(5)–C(10)–C(14) = 109.95(15), N(5)–C(10)–C(18) = 128.6(2), C(14)–C(10)–C(18) = 121.4(2), C(7)–C(11)–C(9) = 111.60(14), C(7)–C(11)–C(21) = 119.7(2), C(9)–C(11)–C(21) = 128.6(2), O(1)–C(12)–C(7) = 126.6(2), O(1)–C(12)–C(16) = 124.62, C(7)–C(12)–C(16) = 108.74, C(8)–C(13)–C(15) = 116.38, C(8)–C(13)–C(16) = 104.90, C(6)–C(14)–C(10) = 109.03(14), C(6)–C(14)–C(17) = 131.4(2), C(10)–C(14)–C(17) = 119.5(2), O(2)–C(15)–C(13) = 118.2(2), O(2)–C(15)–C(25) = 109.49(15), C(13)–C(15)–C(25) = 132.2(2), C(6)–C(16)–C(9) = 114.34, C(6)–C(16)–C(12) = 109.00, C(6)–C(16)–C(13) = 103.43, C(14)–C(17)–C(29) = 119.1(2), C(10)–C(18)–C(27) = 118.0(2), C(7)–C(19)–C(26) = 118.1(2), C(11)–C(21)–C(28) = 117.9(2), C(23)–C(22)–C(25) = 106.9(2), O(2)–C(23)–C(22) = 110.7(2), O(4)–C(24)–N(5) = 125.5(2), O(4)–C(24)–C(6) = 126.03, N(5)–C(24)–C(6) = 108.51, C(15)–C(25)–C(22) = 106.9(2), C(19)–C(26)–C(28) = 119.9(2), C(18)–C(27)–C(29) = 121.4(2), C(21)–C(28)–C(26) = 122.5(2), C(17)–C(29)–C(27) = 120.5(2).

properties were recorded at successive time intervals (1, 2, 3 and 4 h) and compared with that of indomethacin (at a dose of 10 mg/kg body weight) which was used as a reference standard. From the obtained results (Table 2, Figs. 2–4), it has been noticed that many of the tested compounds exhibit considerable anti-inflammatory properties, especially **4a** and **4m** which reveal remarkable activities with potency (percentage

Table 1
Anti-tumor properties of the tested compounds at a dose of 10 μM utilizing human tumor cell lines

Panel/cell line	Percentage growth of tumor cell lines treated with the tested compounds					
	4c	**4d**	**4i**	**4j**	**4k**	**4l**
Non-small cell lung cancer						
EKVX	52.51	72.63	92.41	67.31	97.50	106.93
HOP-62	87.48	88.13	100.21	88.25	110.41	99.87
HOP-92	50.22	**25.15**	62.49	**47.08**	72.90	69.15
NCI-H226	73.70	63.16	85.87	78.69	96.20	85.67
NCI-H23	75.04	57.97	89.26	84.48	92.05	92.03
NCI-H322M	104.22	105.89	111.22	123.90	98.86	89.29
NCI-H460	94.68	67.09	106.76	95.42	109.28	106.96
NCI-H522	73.88	77.27	91.05	87.02	92.30	94.86
Colon cancer						
COLO 205	84.85	70.49	99.95	104.04	119.66	107.18
HCC-2998	95.72	87.99	76.71	103.55	102.66	104.38
HCT-116	54.65	**24.29**	92.61	59.81	92.22	107.98
HCT-15	82.53	68.26	107.42	89.11	103.03	107.77
HT29	73.84	**46.08**	138.35	66.92	99.63	97.52
KM12	81.20	68.71	95.35	98.22	94.28	106.89
SW-620	76.22	67.84	87.52	83.30	89.50	89.09
Breast cancer						
BT-549	121.03	111.20	118.33	101.24	119.40	131.43
HS 578T	**−4.92**	55.19	**16.81**	**−4.27**	69.83	**21.07**
MCF7	109.05	91.83	120.47	99.98	109.43	103.30
MDA-MB-231/ATCC	75.11	79.35	74.44	94.29	78.31	84.32
MDA-MB-435	95.08	100.95	124.31	101.42	115.23	110.92
NCI/ADR-RES	63.99	79.40	89.40	95.95	97.40	98.53
T-47D	81.82	75.79	97.45	93.28	96.05	101.15
Ovarian cancer						
OVCAR-3	78.56	69.31	98.47	92.47	95.59	100.50
OVCAR-4	74.53	72.46	118.37	78.75	92.97	100.02
OVCAR-5	93.22	95.41	106.48	97.71	98.82	101.72
OVCAR-8	69.16	60.94	55.33	83.76	60.21	63.98
SK-OV-3	91.00	86.58	97.35	99.42	96.18	102.91
Leukemia						
CCRF-CEM	74.70	107.80	87.27	72.13	91.38	90.54
HL-60(TB)	76.65	NT[a]	72.80	169.57	89.70	69.98
K-562	58.46	**39.52**	93.86	85.18	93.71	72.50
MOLT-4	57.02	87.51	79.76	106.95	67.72	72.17
RPMI-8226	**36.59**	**13.37**	66.75	**17.73**	89.11	87.94
SR	166.94	51.44	139.65	124.72	60.26	72.54
Renal cancer						
786-0	88.94	86.21	101.98	87.98	110.87	106.00
A498	61.86	82.31	105.20	95.19	101.68	102.56
ACHN	80.46	82.76	98.12	82.76	97.92	98.42
CAKI-1	79.95	71.80	85.74	91.14	106.11	105.78
SN12C	79.51	86.48	105.15	89.10	99.61	104.55
TK-10	144.27	139.50	176.31	165.33	143.73	148.93
UO-31	62.72	66.07	92.55	75.55	95.83	93.46
Melanoma						
LOX IMVI	65.17	72.18	80.52	78.54	79.95	85.29
M14	82.93	76.62	97.55	94.11	103.19	104.42
MALME-3M	120.00	102.09	132.45	103.94	93.48	122.84
SK-MEL-2	**−6.14**	**−11.05**	**8.70**	**−6.92**	**6.58**	**−5.11**
SK-MEL-28	111.60	90.57	108.27	101.34	117.36	105.11
SK-MEL-5	111.87	87.03	101.11	88.14	114.04	112.09
UACC-257	**34.52**	**42.60**	99.29	88.74	64.98	52.59
UACC-62	**45.77**	**48.35**	71.78	63.09	82.03	75.26
Prostate cancer						
DU-145	102.98	91.10	110.06	128.41	92.05	121.40
PC-3	**36.52**	**38.32**	66.02	58.35	72.98	70.28
CNS cancer						
SF-268	89.98	81.09	112.86	107.58	93.56	100.99
SF-295	**31.44**	65.05	103.14	89.09	104.80	60.42

Table 1 (*continued*)

Panel/cell line	Percentage growth of tumor cell lines treated with the tested compounds					
	4c	**4d**	**4i**	**4j**	**4k**	**4l**
SF-539	107.10	90.45	100.65	99.90	108.81	111.67
SNB-19	89.92	89.86	92.18	94.09	99.76	96.25
SNB-75	61.07	75.26	102.06	79.05	80.99	79.00
U251	87.65	93.11	96.72	88.39	100.00	102.48

[a] NT = not tested.

oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin) of 0.81 and 0.69, respectively. The first hour anti-inflammatory data indicate that most of the tested compounds are highly active agents and the activities decrease by time, in contrast with indomethacin (reference standard) which shows more or less constant anti-inflammatory properties at all the detected time intervals.

Structure–activity relationships based on the obtained results indicate that the type of substituents attached to C-4′ is a controlling factor in developing the total pharmacological properties. The best observed anti-inflammatory property is that in which C-4′ is attached to unsubstituted phenyl group. However, substitution of the phenyl group with either electron-withdrawing (chlorine, fluorine) or electron-donating (methyl, methoxy) functions was accompanied with decrease in anti-inflammatory behaviour. It has also been noticed that substitution of the phenyl group with a fluorine residue reflects an enhanced anti-inflammatory property than the case of using a chlorine function, as exhibited in pairs **4e**, **4c** (potency, 0.45, 0.32, respectively) and **4f**, **4d** (potency, 0.53, 0.18, respectively). It has been also noticed that attachment of C-4′ with either thiophene or furan moieties (as examples of heterocyclic rings) led to decrease in the observed pharmacological properties comparable with the case of using unsubstituted phenyl ring. Additionally, it has been observed that compounds possessing a furanyl ring at C-4′ (**4m**, **4n**) show considerably enhanced anti-inflammatory properties "in most detected time intervals" than those substituted with a thienyl moiety (**4k**, **4l**). It has also been noticed that substitution of C-1″ with a methyl group led in most cases to a decrease in the observed anti-inflammatory properties as indicated in pairs **4c**, **4d** (potency, 0.32, 0.18), **4g**, **4h** (potency, 0.35, 0.29), **4i**, **4j** (potency, 0.45, 0.08), **4k**, **4l** (potency, 0.41, 0.27) and **4m**, **4n** (potency, 0.69, 0.17).

From all the above, it could be concluded that although none of the prepared compounds seems superior as anti-tumor or anti-inflammatory active agent, the constructed ring system could be recognized as a hit for developing a better pharmacologically active agent depending on the observed data.

3. Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra (KBr) were recorded on a Nexus 670 FT-IR spectrophotometer. ^{1}H, ^{13}C NMR (of compound **4b**) and HMQC spectra were recorded on a Varian MERCURY 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) spectrometer in $CDCl_3$. Mass spectra were recorded on a Finnigan SSQ 7000 spectrometer (EI, 70 eV). The starting compounds **1a–g** [40,41] were prepared according to the previously reported procedures.

*3.1. Synthesis of dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-diones **4a–n** (general procedure)*

A mixture of the appropriate **1a–g** (5 mmol), **2a**,**b** (5.5 mmol) and sarcosine (5.5 mmol) in absolute ethanol (25 ml) was boiled under reflux for the appropriate time.

Table 2
Anti-inflammatory activity of the tested compounds using acute carrageenan-induced paw oedema in rats

Compound	Mean swelling volume, ml (percentage inhibition of oedema)				Potency[c]
	1 h	2 h	3 h	4 h	
Control	0.527 ± 0.183^{b} (00.0)	0.647 ± 0.074^{b} (00.0)	0.748 ± 0.061^{b} (00.0)	0.850 ± 0.059^{b} (00.0)	–
Indomethacin	0.258 ± 0.010^{a} (51.0)	0.352 ± 0.024^{a} (45.6)	0.387 ± 0.031^{a} (48.3)	0.438 ± 0.030^{a} (48.5)	1.00
4a	0.232 ± 0.025^{a} (56.0)	0.388 ± 0.022^{a} (40.0)	0.430 ± 0.038^{a} (42.5)	0.516 ± 0.023^{a} (39.3)	0.81
4c	0.380 ± 0.035 (27.9)	0.463 ± 0.077^{a} (28.4)	0.607 ± 0.079^{b} (18.9)	0.717 ± 0.044^{b} (15.6)	0.32
4d	0.278 ± 0.059^{a} (47.2)	0.412 ± 0.102^{a} (36.3)	0.678 ± 0.104^{b} (9.4)	0.778 ± 0.082^{b} (8.5)	0.18
4e	0.313 ± 0.028^{a} (40.6)	0.510 ± 0.037^{b} (21.2)	0.590 ± 0.069^{b} (21.1)	$0.665 \pm 0.067^{a,b}$ (21.8)	0.45
4f	0.248 ± 0.032^{a} (52.9)	0.488 ± 0.044^{a} (24.6)	$0.547 \pm 0.050^{a,b}$ (26.9)	$0.633 \pm 0.041^{a,b}$ (25.5)	0.53
4g	0.230 ± 0.049^{a} (56.4)	0.493 ± 0.093^{a} (23.8)	0.543 ± 0.099^{a} (27.4)	0.705 ± 0.084^{b} (17.1)	0.35
4h	0.280 ± 0.060^{a} (46.9)	0.595 ± 0.057^{b} (8.0)	0.700 ± 0.046^{b} (6.4)	0.732 ± 0.034^{b} (13.9)	0.29
4i	0.310 ± 0.027^{a} (41.2)	0.534 ± 0.084^{b} (17.5)	0.595 ± 0.114^{b} (20.5)	$0.665 \pm 0.143^{a,b}$ (21.8)	0.45
4j	0.280 ± 0.042^{a} (46.9)	0.516 ± 0.104^{b} (20.2)	0.723 ± 0.135^{b} (3.3)	0.818 ± 0.111^{b} (3.8)	0.08
4k	0.264 ± 0.031^{a} (49.9)	0.394 ± 0.057^{a} (39.1)	0.504 ± 0.038^{a} (32.6)	$0.680 \pm 0.070^{a,b}$ (20.0)	0.41
4l	0.353 ± 0.038^{a} (33.0)	0.577 ± 0.047^{b} (10.8)	0.680 ± 0.054^{b} (9.1)	0.740 ± 0.044^{b} (12.9)	0.27
4m	0.270 ± 0.028^{a} (48.8)	0.300 ± 0.030^{a} (53.6)	0.458 ± 0.042^{a} (38.8)	0.565 ± 0.038^{a} (33.5)	0.69
4n	0.263 ± 0.022^{a} (50.1)	0.433 ± 0.079^{a} (33.1)	0.617 ± 0.086^{b} (17.5)	0.780 ± 0.068^{b} (8.2)	0.17

[a] Statistically significant from the control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.
[c] Potency was expressed as percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin "reference standard" at 4 h effect.

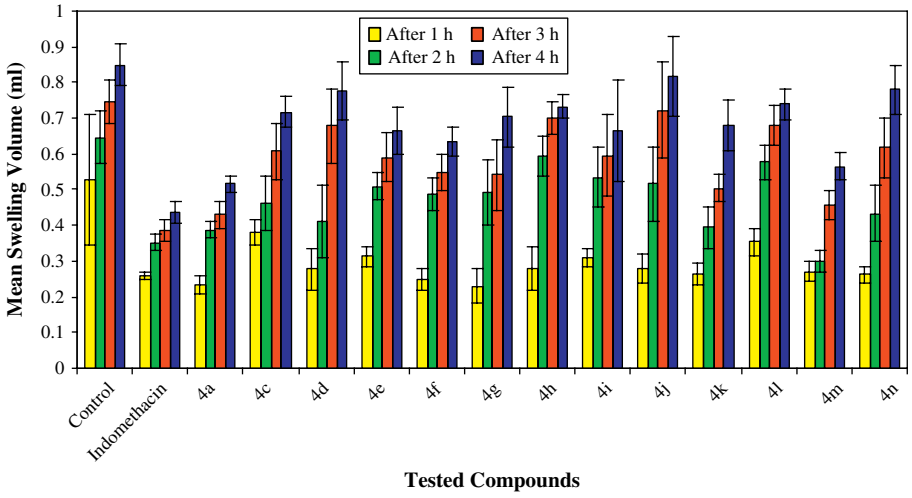


Fig. 2. Mean oedema volume (ml) of the tested compounds at successive time intervals.

The separated solid while refluxing, was collected and crystallized from a suitable solvent affording **4** as colourless crystals. However, in case of **4d**,**f**,**h**,**j**,**m** the reaction mixture was stored to cool at room temperature (20–25 °C) overnight. So, the desired product was separated out which was collected and crystallized from a suitable solvent.

*3.1.1. 2,3-Dihydro-1′-methyl-4′-phenyl-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4a**)*

Reaction time 4 h, crystallizes from *n*-butanol, mp 238–240 °C, yield 86%. IR: ν_{max}/cm^{-1} 3385 (NH), 1710 (br, C=O), 1620, 1467 (C=C). ^{1}H NMR: δ 2.27 (s, 3H, pyrrolidinyl NCH_3), 2.74 (d, 1H, upfield H of indanyl CH_2, $J = 18.0$ Hz), 3.09 (d, 1H, downfield H of indanyl CH_2, $J = 18.0$ Hz), 3.64 (dd, 1H, upfield H of CH_2CH, $J = 8.1$, 9.0 Hz), 4.12 (t, 1H, downfield H of CH_2CH, $J = 9.6$ Hz), 4.39 (dd, 1H, *CH*CH_2, $J = 8.1$, 9.6 Hz), 6.57–7.52 (m, 14H, 13 arom. H + N*H*). MS: *m/z* (%) 395 [(M + 1), 7], 394 (M, 2), 366 (44), 351 (16), 350 (30), 323 (15), 262 (64). Anal. Calcd. for $C_{26}H_{22}N_2O_2$ (394.46): C, 79.16; H, 5.62; N, 7.10. Found: C, 79.42; H, 5.76; N, 7.31.

*3.1.2. 2,3-Dihydro-1′,1″-dimethyl-4′-phenyl-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4b**)*

Reaction time 5 h, crystallizes from *n*-butanol, mp 226–228 °C, yield 93%. IR: ν_{max}/cm^{-1} 1706 (br, C=O), 1608, 1464 (C=C). ^{1}H NMR: δ 2.18 (s, 3H, pyrrolidinyl NCH_3), 2.70 (d, 1H, upfield H of indanyl CH_2, $J = 18.0$ Hz), 2.90 (d, 1H, downfield H of indanyl CH_2, $J = 18.0$ Hz), 3.09 (s, 3H, oxindolyl NCH_3), 3.62 (dd, 1H, upfield H of CH_2CH, $J = 8.1$, 9.0 Hz), 4.13 (t, 1H, downfield H of CH_2CH, $J = 9.6$ Hz), 4.36 (dd, 1H, *CH*CH_2, $J = 8.1$, 9.6 Hz), 6.54–7.49 (m, 13H, arom. H). ^{13}C NMR (APT): δ 25.26 (oxindolyl NCH_3), 34.39 (pyrrolidinyl NCH_3), 34.48 (H_2C-3), 49.36 (*HC*-4′), 58.37 (H_2C-5′), 65.88 [spiro *C*-2 (*C*-3′)], 77.30 [spiro *C*-2′ (*C*-3″)], 108.03, 121.90, 122.93, 125.43, 126.26, 126.82, 127.21, 128.21, 129.04, 129.50, 134.62 (arom. CH), 124.84, 135.00, 138.99, 143.89, 150.94 (arom. quaternary C), 175.53

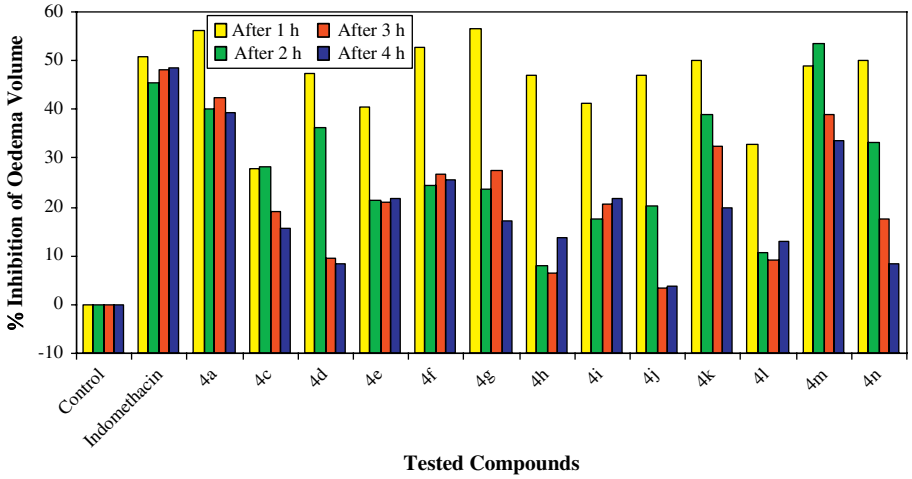


Fig. 3. Percentage inhibition of oedema for the tested compounds at successive time intervals.

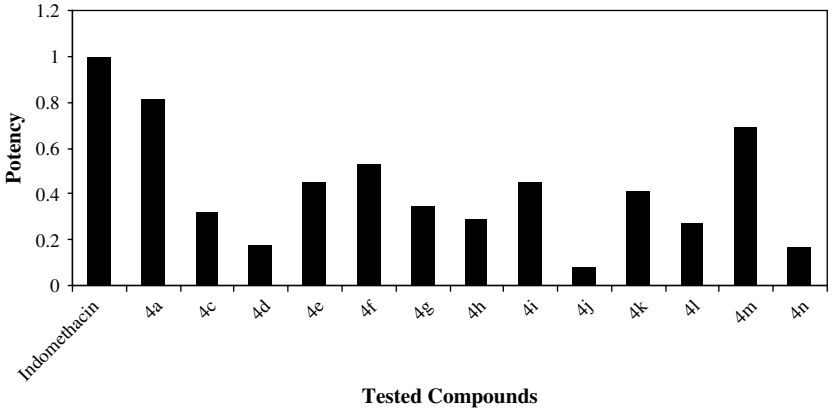


Fig. 4. Anti-inflammatory activity potency of the tested compounds relative to indomethacin which was used as a reference standard.

[oxindolyl C=O (*C*-2″)], 205.44 [indanyl C=O (*C*-1)]. MS: *m/z* (%) 409 [(M + 1), 3], 408 (M, 3), 276 (48). Anal. Calcd. for $C_{27}H_{24}N_2O_2$ (408.48): C, 79.38; H, 5.92; N, 6.86. Found: C, 79.52; H, 6.10; N, 7.09.

*3.1.3. 4′-(4-Chlorophenyl)-2,3-dihydro-1′-methyl-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4c**)*

Reaction time 5 h, crystallizes from *n*-butanol, mp 227–229 °C, yield 75%. IR: ν_{max}/cm^{-1} 3369 (NH), 1718, 1702 (C=O), 1618, 1468 (C=C). ^{1}H NMR: δ 2.25 (s, 3H, pyrrolidinyl NCH_3), 2.70 (d, 1H, upfield H of indanyl CH_2, $J = 18.0$ Hz), 3.06 (d, 1H, downfield H of indanyl CH_2, $J = 18.0$ Hz), 3.63 (t, 1H, upfield H of CH_2CH, $J = 8.4$ Hz), 4.04 (t, 1H, downfield H of CH_2CH, $J = 9.3$ Hz), 4.33 (dd, 1H, *CH*CH_2, $J = 8.4$, 9.6 Hz), 6.58–7.53 (m, 12H, arom. H), 7.63 (s, 1H, N*H*). MS: *m/z* (%) 429 [(M + 1), 3], 428 (M, 5), 400 (40), 385 (12), 384 (15), 357 (11), 262 (87). Anal. Calcd. for $C_{26}H_{21}ClN_2O_2$ (428.903): C, 72.80; H, 4.94; N, 6.53. Found: C, 72.99; H, 5.01; N, 6.69.

*3.1.4. 4′-(4-Chlorophenyl)-2,3-dihydro-1′,1″-dimethyl-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4d**)*

Reaction time 5 h, crystallizes from ethanol, mp 162–164 °C, yield 90%. IR: ν_{max}/cm^{-1} 1705 (br, C=O), 1609, 1469 (C=C). ^{1}H NMR: δ 2.18 (s, 3H, pyrrolidinyl NCH_3), 2.67 (d, 1H, upfield H of indanyl CH_2, $J = 18.0$ Hz), 2.87 (d, 1H, downfield H of indanyl CH_2, $J = 18.0$ Hz), 3.11 (s, 3H, oxindolyl NCH_3), 3.62 (dd, 1H, upfield H of CH_2CH, $J = 8.4$, 9.0 Hz), 4.07 (t, 1H, downfield H of CH_2CH, $J = 9.6$ Hz), 4.31 (dd, 1H, *CH*CH_2, $J = 8.4$, 9.6 Hz), 6.56–7.52 (m, 12H, arom. H). MS: *m/z* (%) 443 [(M + 1), 3], 442 (M, 2), 276 (56). Anal. Calcd. for $C_{27}H_{23}ClN_2O_2$ (442.923): C, 73.21; H, 5.23; N, 6.33. Found: C, 73.42; H, 5.34; N, 6.24.

*3.1.5. 2,3-Dihydro-4′-(4-fluorophenyl)-1′-methyl-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4e**)*

Reaction time 6 h, crystallizes from ethanol, mp 233–235 °C, yield 78%. IR: ν_{max}/cm^{-1} 3415 (NH), 1721, 1703 (C=O), 1615, 1511 (C=C). ^{1}H NMR: δ 2.26 (s, 3H, pyrrolidinyl NCH_3), 2.71 (d, 1H, upfield H of indanyl CH_2, $J = 17.7$ Hz), 3.05 (d, 1H, downfield H of indanyl CH_2, $J = 17.7$ Hz), 3.64 (dd, 1H, upfield H of CH_2CH, $J = 8.4$, 9.0 Hz), 4.05 (t, 1H, downfield H of CH_2CH, $J = 9.6$ Hz), 4.35 (dd, 1H, *CH*CH_2, $J = 8.4$, 9.6 Hz), 6.60–7.54 (m, 12H, arom. H), 7.78 (s, 1H, N*H*). MS: *m/z* (%) 413 [(M + 1), 4], 412 (M, 4), 384 (23), 369 (3), 368 (11), 341 (9), 262 (75). Anal. Calcd. for $C_{26}H_{21}FN_2O_2$ (412.45): C, 75.71; H, 5.13; N, 6.79. Found: C, 75.82; H, 5.19; N, 6.83.

*3.1.6. 2,3-Dihydro-1′,1″-dimethyl-4′-(4-fluorophenyl)-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4f**)*

Reaction time 9 h, crystallizes from *n*-butanol, mp 208–210 °C, yield 80%. IR: ν_{max}/cm^{-1} 1711, 1699 (C=O), 1607, 1512 (C=C). ^{1}H NMR: δ 2.18 (s, 3H, pyrrolidinyl NCH_3), 2.68 (d, 1H, upfield H of indanyl CH_2, $J = 17.7$ Hz), 2.87 (d, 1H, downfield H of indanyl CH_2, $J = 17.7$ Hz), 3.11 (s, 3H, oxindolyl NCH_3), 3.63 (dd, 1H, upfield H of CH_2CH, $J = 8.1$, 8.7 Hz), 4.08 (t, 1H, downfield H of CH_2CH, $J = 9.6$ Hz), 4.32 (dd, 1H, *CH*CH_2, $J = 8.1$, 9.6 Hz), 6.56–7.52 (m, 12H, arom. H). MS: *m/z* (%) 427 [(M + 1), 4], 426 (M, 2), 276 (36). Anal. Calcd. for $C_{27}H_{23}FN_2O_2$ (426.47): C, 76.04; H, 5.44; N, 6.57. Found: C, 75.82; H, 5.28; N, 6.49.

*3.1.7. 2,3-Dihydro-1′-methyl-4′-(4-methylphenyl)-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4g**)*

Reaction time 10 h, crystallizes from *n*-butanol, mp 236–238 °C, yield 83%. IR: ν_{max}/cm^{-1} 3400 (NH), 1717, 1701 (C=O), 1616, 1466 (C=C). ^{1}H NMR: δ 2.26 (s, 3H, pyrrolidinyl NCH_3), 2.30 (s, 3H, Ar–CH_3), 2.76 (d, 1H, upfield H of indanyl CH_2, $J = 18.0$ Hz), 3.09 (d, 1H, downfield H of indanyl CH_2, $J = 18.0$ Hz), 3.61 (dd, 1H, upfield H of CH_2CH, $J = 8.4$, 9.0 Hz), 4.09 (t, 1H, downfield H of CH_2CH, $J = 9.0$ Hz), 4.36 (t, 1H, *CH*CH_2, $J = 8.4$ Hz), 6.58–7.52 (m, 12H, arom. H), 7.69 (s, 1H, N*H*). MS: *m/z* (%) 409 [(M + 1), 8], 408 (M, 3), 380 (24), 365 (14), 364 (25), 337 (13), 262 (55). Anal. Calcd. for $C_{27}H_{24}N_2O_2$

(408.48): C, 79.38; H, 5.92; N, 6.86. Found: C, 79.29; H, 5.81; N, 7.03.

3.1.8. 2,3-Dihydro-1′,1″-dimethyl-4′-(4-methylphenyl)-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4h**)

Reaction time 7 h, crystallizes from methanol, mp 172–174 °C, yield 71%. IR: ν_{max}/cm^{-1} 1708 (br, C=O), 1610, 1473 (C=C). ^{1}H NMR: δ 2.19 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.30 (s, 3H, Ar–C*H*$_3$), 2.74 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 17.7$ Hz), 2.92 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 17.7$ Hz), 3.10 (s, 3H, oxindolyl NC*H*$_3$), 3.61 (dd, 1H, upfield H of C*H*$_2$CH, $J = 8.1$, 9.0 Hz), 4.12 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.9$ Hz), 4.35 (dd, 1H, *CH*CH$_2$, $J = 8.4$, 9.9 Hz), 6.55–7.50 (m, 12H, arom. H). MS: *m/z* (%) 423 [(M + 1), 11], 422 (M, 6), 276 (41). Anal. Calcd. for $C_{28}H_{26}N_2O_2$ (422.51): C, 79.59; H, 6.20; N, 6.63. Found: C, 79.47; H, 6.10; N, 6.50.

3.1.9. 2,3-Dihydro-4′-(4-methoxyphenyl)-1′-methyl-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4i**)

Reaction time 12 h, crystallizes from *n*-butanol, mp 221–223 °C, yield 80%. IR: ν_{max}/cm^{-1} 3404 (NH), 1722 (br, C=O), 1611, 1513 (C=C). ^{1}H NMR: δ 2.26 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.77 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 17.7$ Hz), 3.06 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 17.7$ Hz), 3.62 (t, 1H, upfield H of C*H*$_2$CH, $J = 8.7$ Hz), 3.77 (s, 3H, OC*H*$_3$), 4.06 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.6$ Hz), 4.33 (dd, 1H, *CH*CH$_2$, $J = 8.7$, 9.6 Hz), 6.59–7.53 (m, 12H, arom. H), 7.75 (s, 1H, N*H*). MS: *m/z* (%) 425 [(M + 1), 2], 424 (M, 1), 396 (5), 381 (1), 380 (2), 353 (1), 262 (18). Anal. Calcd. for $C_{27}H_{24}N_2O_3$ (424.48): C, 76.39; H, 5.70; N, 6.60. Found: C, 76.17; H, 5.61; N, 6.78.

3.1.10. 2,3-Dihydro-1′,1″-dimethyl-4′-(4-methoxyphenyl)-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4j**)

Reaction time 12 h, crystallizes from methanol, mp 150–152 °C, yield 87%. IR: ν_{max}/cm^{-1} 1704 (br, C=O), 1609, 1515 (C=C). ^{1}H NMR: δ 2.19 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.74 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 2.88 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.10 (s, 3H, oxindolyl NC*H*$_3$), 3.61 (dd, 1H, upfield H of C*H*$_2$CH, $J = 8.1$, 8.7 Hz), 3.77 (s, 3H, OC*H*$_3$), 4.09 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.6$ Hz), 4.31 (dd, 1H, *CH*CH$_2$, $J = 8.1$, 9.6 Hz), 6.56–7.51 (m, 12H, arom. H). MS: *m/z* (%) 439 [(M + 1), 4], 438 (M, 5), 276 (38). Anal. Calcd. for $C_{28}H_{26}N_2O_3$ (438.51): C, 76.69; H, 5.98; N, 6.39. Found: C, 76.82; H, 6.06; N, 6.45.

3.1.11. 2,3-Dihydro-1′-methyl-4′-(2-thienyl)-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4k**)

Reaction time 20 h, crystallizes from *n*-butanol, mp 226–228 °C, yield 70%. IR: ν_{max}/cm^{-1} 3385 (NH), 1727, 1700 (C=O), 1615, 1466 (C=C). ^{1}H NMR: δ 2.26 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.87 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.08 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.74 (dd, 1H, upfield H of C*H*$_2$CH, $J = 8.7$, 9.0 Hz), 4.10 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.3$ Hz), 4.62 (t, 1H, *CH*CH$_2$, $J = 8.7$ Hz), 6.63–7.58 (m, 11H, arom. H), 7.87 (s, 1H, N*H*). MS: *m/z* (%) 401 [(M + 1), 3], 400 (M, 4), 372 (15), 357 (3), 356 (6), 329 (6), 262 (76). Anal. Calcd. for $C_{24}H_{20}N_2O_2S$ (400.484): C, 71.97; H, 5.03; N, 7.00. Found: C, 72.05; H, 5.07; N, 7.21.

3.1.12. 2,3-Dihydro-1′,1″-dimethyl-4′-(2-thienyl)-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4l**)

Reaction time 14 h, crystallizes from *n*-butanol, mp 211–213 °C, yield 72%. IR: ν_{max}/cm^{-1} 1705 (br, C=O), 1607, 1462 (C=C). ^{1}H NMR: δ 2.19 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.84 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 2.91 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.10 (s, 3H, oxindolyl NC*H*$_3$), 3.73 (t, 1H, upfield H of C*H*$_2$CH, $J = 8.7$ Hz), 4.13 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.3$ Hz), 4.60 (t, 1H, *CH*CH$_2$, $J = 9.0$ Hz), 6.59–7.57 (m, 11H, arom. H). MS: *m/z* (%) 415 [(M + 1), 5], 414 (M, 2), 276 (54). Anal. Calcd. for $C_{25}H_{22}N_2O_2S$ (414.514): C, 72.43; H, 5.35; N, 6.76. Found: C, 72.23; H, 5.18; N, 6.88.

3.1.13. 2,3-Dihydro-4′-(2-furanyl)-1′-methyl-dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4m**)

Reaction time 20 h, crystallizes from methanol, mp 202–204 °C, yield 73%. IR: ν_{max}/cm^{-1} 3183 (NH), 1708, 1695 (C=O), 1620, 1470 (C=C). ^{1}H NMR: δ 2.24 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.73 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.13 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.63 (t, 1H, upfield H of C*H*$_2$CH, $J = 8.7$ Hz), 4.06 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.0$ Hz), 4.41 (t, 1H, *CH*CH$_2$, $J = 8.7$ Hz), 6.31–7.59 (m, 11H, arom. H), 8.05 (s, 1H, N*H*). MS: *m/z* (%) 385 [(M + 1), 100], 384 (M, 7), 356 (39), 341 (2), 340 (6), 313 (5), 262 (46). Anal. Calcd. for $C_{24}H_{20}N_2O_3$ (384.42): C, 74.98; H, 5.24; N, 7.29. Found: C, 74.90; H, 5.15; N, 7.12.

3.1.14. 2,3-Dihydro-1′,1″-dimethyl-4′-(2-furanyl)-dispiro-[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)-dione (**4n**)

Reaction time 20 h, crystallizes from ethanol, mp 185–187 °C, yield 70%. IR: ν_{max}/cm^{-1} 1709 (br, C=O), 1605, 1466 (C=C). ^{1}H NMR: δ 2.17 (s, 3H, pyrrolidinyl NC*H*$_3$), 2.72 (d, 1H, upfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 2.94 (d, 1H, downfield H of indanyl C*H*$_2$, $J = 18.0$ Hz), 3.08 (s, 3H, oxindolyl NC*H*$_3$), 3.63 (t, 1H, upfield H of C*H*$_2$CH, $J = 8.7$ Hz), 4.08 (t, 1H, downfield H of C*H*$_2$CH, $J = 9.0$ Hz), 4.38 (t, 1H, *CH*CH$_2$, $J = 9.0$ Hz), 6.31–7.58 (m, 11H, arom. H). MS: *m/z* (%) 399 [(M + 1), 32], 276 (39). Anal. Calcd. for $C_{25}H_{22}N_2O_3$ (398.45): C, 75.35; H, 5.57; N, 7.03. Found: C, 75.24; H, 5.51; N, 6.96.

3.2. Single crystal X-ray crystallographic data of **4n**

For X-ray crystallographic studies, compound **4n** was recrystallized as prismatic colourless crystals from ethanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo Kα radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR92 [42] and refined by maXus [43] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{25}H_{22}N_2O_3$, $M_r = 398.462$, monoclinic, crystallizes in space group $P2_1/c$, cell lengths $a = 12.2417(3)$, $b = 10.8165(3)$, $c = 15.6072(5)$ Å, cell angles $\alpha = 90.00°$, $\beta = 102.9309(11)°$, $\gamma = 90.00°$'', $V = 2014.18(10)$ Å^3, $Z = 4$, $D_c = 1.314$ mg/m^3, θ values 2.910–27.485°, absorption coefficient μ (Mo $K\alpha$) = 0.09 mm^{-1}, $F(000) = 840$. The unique reflections measured were 5065 of which 2813 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 271 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000F_o^2]$. The final agreement factors were $R = 0.046$ and $wR = 0.082$ with a goodness-of-fit of 1.337. Full crystallographic details, excluding structure factors have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 676720.

3.3. Anti-tumor activity screening

Anti-tumor activity screening for the synthesized compounds (**4c**,**d**,**i**–**l**) at a dose of 10 μM utilizing 56 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney was carried out using adriamycin as a reference standard according to the previously reported standard procedure [20,36–38]. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated in 96-well-microtiter plates in 100 μl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental tested compounds. After 24 h, two plates of each cell lines are fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of tested compound addition (time zero, T_z). Experimental tested compounds are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of the tested compound addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Aliquots of 100 μl of the tested compound dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final concentrations.

Following the tested compound addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Table 1 represents the observed percentage growth of each cell line treated with a certain tested compound relative to control cell line experiments.

3.4. Anti-inflammatory activity screening

The anti-inflammatory activity screening for the prepared compounds was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats [22–24,39]. Wister albino rats of either sex (pregnant female animals were excluded) weighing 160–190 g were divided into 15 groups of 6 animals each. Administration of indomethacin (reference standard in a dose of 10 mg/kg body weight) and the tested compounds (**4a**,**c**–**n**) dissolved in DMSO, in a dose of 50 mg/kg (body weight) was done intraperitoneally 1 h before induction of inflammation. The control group was given DMSO only. Carrageenan paw oedema was induced by subcutaneous injection of 1% solution of carrageenan in saline (0.1 ml/rat) into the right hind paw of rats. Paw volumes were measured volumetrically after successive time intervals (1, 2, 3 and 4 h) with plethysmometer 7150 (UGO BASILE, Italy) and compared with the initial hind paw volume of each rat for determining the oedema volume. Data were collected, checked, revised and analyzed. Quantitative variables from normal distribution were expressed as means ± SE ''standard error''. The significant difference between groups was tested by using one-way ANOVA followed by post hoc test and the chosen level of significance was $p < 0.05$.

The anti-inflammatory activity was expressed as percentage inhibition of oedema volume in treated animals in comparison with the control group (Table 2, Figs. 2–4).

$$\text{Percentage inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

where, V_c and V_t are the volumes of oedema for the control and drug-treated animal groups, respectively.

Potency of the tested compounds was calculated relative to indomethacin ''reference standard'' treated group according to the following equation:

$$\text{Potency} = \frac{\text{Percentage oedema inhibition of tested compound treated group}}{\text{Percentage oedema inhibition of indomethacin treated group}}$$

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