



Original article

Regioselective synthesis and stereochemical structure of anti-tumor active dispiro[3*H*-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1*H*),4''-diones

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Anti-tumor

Anti-inflammatory

ABSTRACT

Reaction of 3,5-bis(arylmethylene)-1-methyl-4-piperidinones **1a–1g** with azomethine ylides (generated in situ via decarboxylative condensation of isatins **2a,2b** with sarcosine **3**) in refluxing ethanol afforded 4'-aryl-5''-(arylmethylene)-dispiro[3*H*-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1*H*),4''-diones **4a–4m** as the sole product in a high regioselective manner. Anti-tumor activity screening of **4e,4f,4k,4m**, as representative examples of the synthesized compounds, at a dose of 10 μ M utilizing 59 different human tumor cell lines representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney exhibited that, the tested compounds reflect mild activity against most of the used human tumor cells. Meanwhile, all the tested compounds reveal considerable anti-tumor properties against colon (HCT-116), breast (T-47D), leukemia [HL-60 (TB), MOLT-4, RPMI-8226] and prostate (PC-3) cancers. Anti-inflammatory properties of the prepared compounds (at a dose of 50 mg/kg body weight) using in vivo acute carrageenan-induced paw oedema in rats exhibited that all the tested compounds possess considerable anti-inflammatory activity especially **4a,4k,4l** which reveal remarkable activities with potency 125.5, 139.3 and 126.4, respectively, relative to indomethacin which was used as a reference standard (at a dose of 10 mg/kg body weight).

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1. Introduction

Piperidin-4-ones represent an important class of bio-active heterocycles attracting a progressive interest of many researchers due to their wide observed biological and pharmacological properties. The skeletal ring of piperidine nucleus is often found in the molecular framework of many natural medicaments [1]. Many 2,6-diarylpiperidin-4-ones were reported as antibacterial and antifungal active agents [2,3]. Moreover, 3,5-bis(arylmethylene)-4-piperidinones were reported to exhibit cytotoxic properties against murine P388 and L1210 leukemic cells as well as human MOLT 4/C8 and CEM T-lymphocytes [4–6], beside their selective toxicity against malignant cells namely, squamous cell carcinomas (HSC-2, HSC-4) and a promyelocytic leukemia neoplasm (HL-60) [7].

In the present work, it is intended to investigate the reaction of azomethine ylides (generated in situ via decarboxylative condensation of α -amino acid, exemplified by sarcosine and isatins, following the previously described successful methods [8–11]) with the exocyclic olefinic linkages derived from 3,5-bis(arylmethylene)-4-piperidinones attempting to isolate spiropyrrolidinyl-oxindolyl system attached to the piperidinyl nucleus. Regioselectivity of the

reaction and stereochemical structure of the target products will be considered during this study. The anti-tumor as well as anti-inflammatory properties of the prepared compounds will be screened. The interest for construction of this combination in the aimed synthesized heterocyclic system is also attributed to the fact that, spiropyrrolidinyl-oxindolyl nucleus represents the main skeleton of many naturally occurring alkaloids such as elacomine [12], horsfiline [13–18] mitraphylline [19] formosanine and rychnofilline [20] which were established to be highly pronounced pharmacological important agents. Additionally, many structurally related analogues were synthesized exhibiting potent, selective inhibitory properties of MDM2-p53 [21]. Recently, 3,3'-dispiropyrrolidinyl-oxindoles have been reported via [3 + 2] cycloaddition of azomethine ylides with isomerised Morita–Baylis–Hillman adducts [22].

2. Results and discussion

2.1. Chemistry

Reaction of 3,5-bis(arylmethylene)-1-methyl-4-piperidinones **1a–1g** with azomethine ylides (generated in situ via decarboxylative condensation of isatins **2a,2b** with sarcosine **3**) in refluxing ethanol afforded only one product as indicated by TLC. The structure of

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which was established to be 4'-aryl-5''-(arylmethylene)-dispiro[3*H*-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1*H*),4''-dione **4** rather than 3'-aryl-5''-(arylmethylene)-dispiro[3*H*-indole-3,2'-pyrrolidine-4',3''-piperidine]-2(1*H*),4''-dione **5**, based on spectroscopic (IR, ¹H NMR, ¹H,¹H-COSY) and elemental analysis data, explaining the high regioselective manner of this reaction (Scheme 1). The IR spectra of **4** reveal the presence of strong carbonyl bands at $\nu = 1706\text{--}1674\text{ cm}^{-1}$ region assignable for piperidinyll ketonic and indolyl amidic carbonyl functions, excluding any cycloaddition reaction taking place with these moieties.

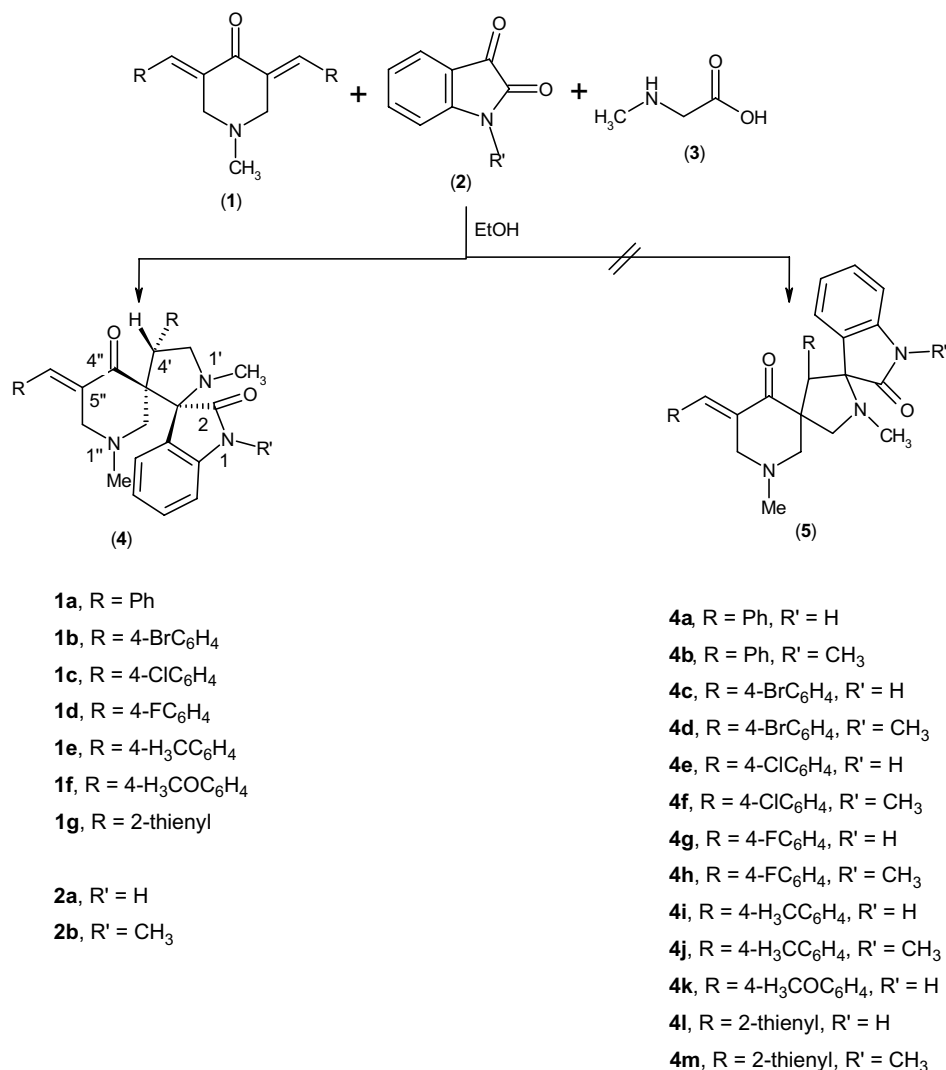
¹H NMR spectra of **4** seem very interesting. They reveal the piperidinyll upfield proton of H₂C-2'' as a doublet signal at $\delta = 1.59\text{--}1.91$ ($J = 10.8\text{--}12.9$ Hz). However, the piperidinyll downfield proton of H₂C-2'' is overlapped with the piperidinyll downfield proton of H₂C-6'' at $\delta = 3.20\text{--}3.36$. The drastic shifting effect of the upfield proton of H₂C-2'' is probably attributed to the anisotropic effect of the oxindolyl nucleus on this neighbouring proton. Otherwise, the piperidinyll upfield proton of H₂C-6'' is appeared as a double doublet signal at $\delta = 2.83\text{--}2.96$, due to its mutual coupling with the geminal proton of H₂C-6'' ($J = 14.4\text{--}15.9$ Hz) and long-range allylic coupling with the exocyclic methine olefinic linkage attached to the piperidinyll C-5'' ($J = 2.4\text{--}2.7$ Hz), which is usually hidden under the aromatic proton region. ¹H,¹H-COSY of **4m** "as a representative example" (Fig. 1) supports these interpretations. The observed

upfield shifting behaviour of H₂C-6'' upfield proton could be attributed to the anisotropic effects of the aryl group attached to the exocyclic olefinic moiety. On the other hand, the pyrrolidinyll H₂C-5' appears as two non-magnetically equivalent protons at $\delta = 3.20\text{--}3.45$, $3.75\text{--}4.01$ coupled with each other and in turn with the vicinal methine proton HC-4' " $\delta = 4.75\text{--}5.06$ " ($J_{gem} = 8.4\text{--}9.0$, $J_{vic} = 6.9\text{--}9.3$, $9.0\text{--}11.4$ Hz). Single crystal X-ray diffraction of **4k** (Fig. 2) supports the established structure indicating that the isolated crystalline form product is 3*R*, 3''*S*, 4'*R*. In addition, the piperidinyll nucleus is observed as a distorted chair-form configuration. While, the geometrical configuration of the exocyclic olefinic linkage attached to the piperidinyll C-5'' is still preserved (*E*-form configuration). In other words, there are two spiro junctions in the molecule, which consists of a nearly planar 2-oxindole ring, an envelope pyrrolidine ring and a chair piperidone ring comparable to other recently reported X-ray data of a similar analogue [23].

Attempts have been made towards the preparation of dicycloaddition products utilizing both exocyclic olefinic linkages of **1** via reaction of **1** with **2** and **3** in 1:2:2 molar ratio, but were unsuccessful.

2.2. Anti-tumor activity

Anti-tumor activity screening of **4e**, **4f**, **4k**, **4m**, as representative examples of the synthesized compounds, adopted by



Scheme 1.

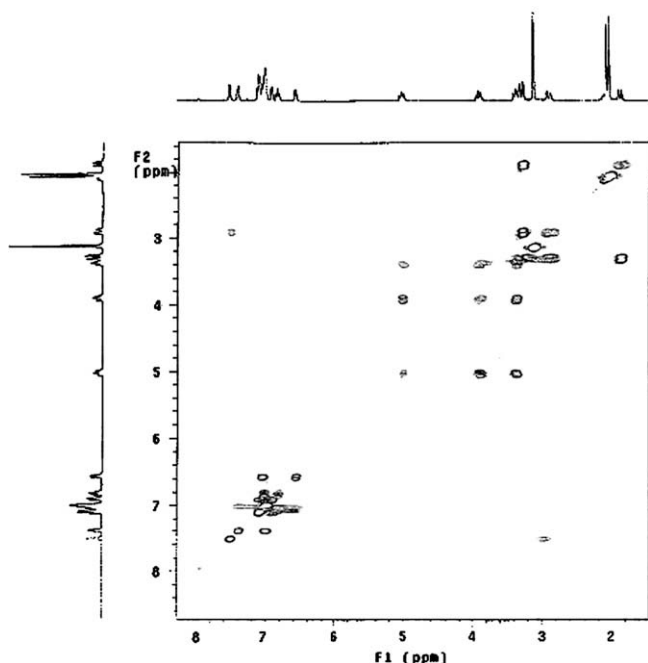


Fig. 1. ^1H , ^1H -COSY spectrum of **4m**.

developmental therapeutics program of National Cancer Institute (Bethesda, Maryland, USA), at a dose of 10 μM utilizing 59 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 [24–27]. 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 mild activity against most of the used human tumor cells. However, all the tested compounds reveal considerable anti-tumor properties against colon (HCT-116), breast (T-47D), leukemia [HL-60(TB), MOLT-4, RPMI-8226] and prostate (PC-3) cancers, considering cell line growth inhibition with >50% at a concentration of 10 μM usually seems a noticeable activity. Structure–activity relationships based on the observed data explain that the substitution attached to the phenyl group oriented at the 4'-position of the synthesized heterocycle plays an important role in developing the observed anti-tumor properties. It has been noticed that, adoption of a methoxy function (an electron-donating group) as a substitution attached to the phenyl group at this position seems more preferable for constructing an anti-tumor active agent than in the case of using a chlorine residue (deactivating moiety). Also, adoption of a thienyl function (which could be recognized as a bio-isostere of the phenyl group) at the 4'-position of the constructed heterocycle seems, in many cases, a successful choice for designing an anti-tumor active agent.

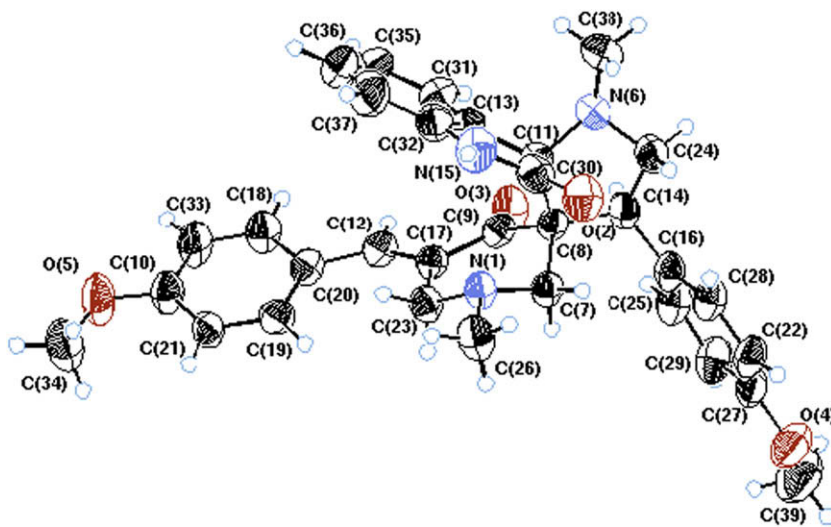


Fig. 2. ORTEP projection of single crystal X-ray diffraction of **4k**. Selected intramolecular bond lengths (Å) and bond angles ($^\circ$) of **4k**. N(1)–C(7) = 1.450(2), N(1)–C(23) = 1.453(3), N(1)–C(26) = 1.452(3), O(2)–C(30) = 1.239(3), O(3)–C(9) = 1.222(2), O(4)–C(27) = 1.379(3), O(4)–C(39) = 1.403(4), O(5)–C(10) = 1.358(3), O(5)–C(34) = 1.415(3), N(6)–C(11) = 1.482(3), N(6)–C(24) = 1.448(3), N(6)–C(38) = 1.449(3), C(7)–C(8) = 1.522(3), C(8)–C(9) = 1.531(3), C(8)–C(11) = 1.596(3), C(8)–C(14) = 1.562(3), C(9)–C(17) = 1.484(3), C(10)–C(21) = 1.371(3), C(10)–C(33) = 1.379(3), C(11)–C(13) = 1.516(3), C(11)–C(30) = 1.544(3), C(12)–C(17) = 1.341(3), C(12)–C(20) = 1.465(3), C(13)–C(31) = 1.380(3), C(13)–C(32) = 1.387(3), C(14)–C(16) = 1.512(3), C(14)–C(24) = 1.523(3), N(15)–C(30) = 1.351(3), N(15)–C(32) = 1.397(3), C(16)–C(25) = 1.383(3), C(16)–C(28) = 1.388(3), C(17)–C(23) = 1.503(3), C(18)–C(20) = 1.391(3), C(18)–C(33) = 1.371(3), C(19)–C(20) = 1.396(3), C(19)–C(21) = 1.377(3), C(22)–C(27) = 1.378(3), C(22)–C(28) = 1.375(3), C(25)–C(29) = 1.386(3), C(27)–C(29) = 1.372(3), C(31)–C(35) = 1.378(3), C(32)–C(37) = 1.391(3), C(35)–C(36) = 1.377(4), C(36)–C(37) = 1.372(4), C(7)–H(7A) = 0.960(2), C(7)–H(7B) = 0.960(2), C(12)–H(12) = 0.960(2), C(14)–H(14) = 0.960(2), N(15)–H(15) = 0.960(2), C(23)–H(23A) = 0.960(2), C(23)–H(23B) = 0.960(2), C(24)–H(24A) = 0.960(2), C(24)–H(24B) = 0.960(2), C(7)–N(1)–C(23) = 111.5(2), C(7)–N(1)–C(26) = 113.5(2), C(23)–N(1)–C(26) = 110.6(2), C(27)–O(4)–C(39) = 117.7(2), C(10)–O(5)–C(34) = 118.7(2), C(11)–N(6)–C(24) = 106.7(2), C(11)–N(6)–C(38) = 115.8(2), C(24)–N(6)–C(38) = 115.9(2), N(1)–C(7)–C(8) = 108.29(15), C(7)–C(8)–C(9) = 105.8(2), C(7)–C(8)–C(11) = 111.7(2), C(7)–C(8)–C(14) = 114.52(15), C(9)–C(8)–C(11) = 109.24(15), C(9)–C(8)–C(14) = 111.7(2), C(11)–C(8)–C(14) = 104.0(2), O(3)–C(9)–C(8) = 120.6(2), O(3)–C(9)–C(17) = 121.5(2), C(8)–C(9)–C(17) = 117.9(2), O(5)–C(10)–C(21) = 125.1(2), O(5)–C(10)–C(33) = 115.6(2), C(21)–C(10)–C(33) = 119.3(2), N(6)–C(11)–C(8) = 102.6(2), N(6)–C(11)–C(13) = 110.7(2), N(6)–C(11)–C(30) = 112.5(2), C(8)–C(11)–C(13) = 117.5(2), C(8)–C(11)–C(30) = 113.0(2), C(13)–C(11)–C(30) = 101.0(2), C(17)–C(12)–C(20) = 132.5(2), C(11)–C(13)–C(31) = 131.4(2), C(11)–C(13)–C(32) = 108.9(2), C(31)–C(13)–C(32) = 119.7(2), C(8)–C(14)–C(16) = 116.7(2), C(8)–C(14)–C(24) = 103.7(2), C(16)–C(14)–C(24) = 116.5(2), C(30)–N(15)–C(32) = 111.3(2), C(14)–C(16)–C(25) = 119.5(2), C(14)–C(16)–C(28) = 123.7(2), C(25)–C(16)–C(28) = 116.9(2), C(9)–C(17)–C(12) = 115.9(2), C(9)–C(17)–C(23) = 119.4(2), C(12)–C(17)–C(23) = 124.6(2), C(20)–C(18)–C(33) = 122.2(2), C(20)–C(19)–C(21) = 121.9(2), C(12)–C(20)–C(18) = 117.5(2), C(12)–C(20)–C(19) = 126.3(2), C(18)–C(20)–C(19) = 116.2(2), C(10)–C(21)–C(19) = 120.3(2), C(27)–C(22)–C(28) = 119.9(2), N(1)–C(23)–C(17) = 113.3(2), N(6)–C(24)–C(14) = 102.2(2), C(16)–C(25)–C(29) = 121.9(2), O(4)–C(27)–C(22) = 114.9(2), O(4)–C(27)–C(29) = 125.5(2), C(22)–C(27)–C(29) = 119.7(2), C(16)–C(28)–C(22) = 121.9(2), C(25)–C(29)–C(27) = 119.6(2), O(2)–C(30)–N(15) = 125.8(2), O(2)–C(30)–N(15) = 125.1(2), C(11)–C(30)–N(15) = 108.9(2), C(13)–C(31)–C(35) = 119.3(2), C(13)–C(32)–N(15) = 109.9(2), C(13)–C(32)–C(37) = 121.0(3), N(15)–C(32)–C(37) = 129.0(2), C(10)–C(33)–C(18) = 120.1(2), C(31)–C(35)–C(36) = 120.6(3), C(35)–C(36)–C(37) = 121.1(2), C(32)–C(37)–C(36) = 118.2(3), C(17)–C(12)–H(12) = 119.7(2), C(20)–C(12)–H(12) = 107.8(2), C(8)–C(14)–H(14) = 104.9(2), C(16)–C(14)–H(14) = 108.8(2), C(24)–C(14)–H(14) = 105.1(2).

Table 1

Anti-tumor properties of the tested compounds at a dose of 10 μ M utilizing human tumor cell lines

Panel/cell line	Percentage growth of tumor cell lines treated with the tested compounds			
	4e	4f	4k	4m
<i>Non-small cell lung cancer</i>				
A549/ATCC	62.41	70.05	44.42	58.00
EKVX	37.21	42.06	64.22	47.12
HOP-62	73.57	71.03	69.93	58.81
HOP-92	56.24	61.64	46.67	41.11
NCI-H226	64.42	58.65	63.02	58.03
NCI-H23	44.78	33.92	50.40	26.09
NCI-H322M	68.66	79.10	79.70	74.98
NCI-H460	66.71	71.55	52.34	52.12
NCI-H522	59.82	56.35	50.17	49.42
<i>Colon cancer</i>				
COLO 205	92.67	99.60	67.43	69.01
HCC-2998	70.66	71.88	65.45	40.58
HCT-116	45.85	29.87	26.75	30.60
HCT-15	68.53	76.25	50.76	62.11
HT29	59.51	68.36	58.14	56.06
KM12	64.22	78.70	37.71	45.22
SW-620	57.67	68.11	47.82	53.81
<i>Breast cancer</i>				
BT-549	63.93	80.33	56.29	79.74
HS 578T	79.70	97.29	74.71	79.30
MCF7	44.88	86.39	30.87	50.43
MDA-MB-231/ATCC	74.89	70.38	59.41	52.35
MDA-MB-435	61.11	87.85	60.67	40.58
MDA-MB-468	56.56	66.86	58.94	33.40
NCI/ADR-RES	62.13	56.88	36.54	37.71
T-47D	46.15	46.66	31.58	22.67
<i>Ovarian cancer</i>				
IGROV1	81.75	92.12	79.38	81.40
OVCAR-3	48.43	60.54	50.31	33.60
OVCAR-4	49.57	43.09	52.68	37.29
OVCAR-5	94.16	87.70	92.45	103.35
OVCAR-8	69.38	79.45	52.38	65.70
SK-OV-3	80.63	85.23	75.58	59.45
<i>Leukemia</i>				
CCRF-CEM	51.28	31.54	45.39	56.42
HL-60(TB)	41.60	16.65	21.49	22.57
K-562	45.08	30.39	73.33	59.47
MOLT-4	37.40	43.22	17.19	27.16
RPMI-8226	38.54	38.04	20.74	10.58
SR	67.53	70.31	52.27	30.48
<i>Renal cancer</i>				
786-0	71.82	73.88	57.17	65.76
A498	55.26	69.44	32.51	46.47
ACHN	63.56	57.48	49.10	56.46
CAKI-1	45.81	56.61	54.30	57.25
RXF 393	67.45	67.15	57.78	55.61
SN12C	64.08	62.91	58.37	64.34
TK-10	88.59	81.80	73.10	78.85
UO-31	43.20	56.58	40.44	45.37
<i>Melanoma</i>				
LOX IMVI	72.29	73.87	65.34	64.95
M14	78.32	77.85	55.64	58.45
MALME-3M	91.87	73.51	58.74	76.40
SK-MEL-2	76.22	71.88	60.68	68.62
SK-MEL-28	101.13	104.04	76.31	80.00
UACC-257	79.26	71.98	70.31	64.56
UACC-62	67.23	59.87	47.75	47.62
<i>Prostate cancer</i>				
DU-145	83.82	88.42	70.31	67.36
PC-3	24.86	32.53	21.04	17.75
<i>CNS cancer</i>				
SF-268	60.57	83.61	61.78	72.42
SF-295	64.52	65.59	NT ^a	NT ^a
SF-539	83.30	78.12	73.98	75.43
SNB-19	76.50	77.33	61.58	65.29
SNB-75	56.70	65.15	59.37	64.52
U251	64.76	68.64	52.08	62.77

^a NT = not tested.

2.3. Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds **4a–4m** (at a dose of 50 mg/kg body weight) was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats [28–31]. The anti-inflammatory properties were recorded at successive time intervals (1,2,3,4 and 24 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. 3–5), it has been noticed that, all the synthesized compounds exhibit anti-inflammatory properties especially, **4a–4c,4g,4j–4m** which reveal remarkable activities with potency (% oedema inhibition of the tested compounds relative to % oedema inhibition of indomethacin) 125.5, 121.8, 118.2, 108.8, 121.8, 139.3, 126.4 and 123.5 respectively, i.e. they seem to be more potent than the commonly used standard drug indomethacin. It has also been noticed that, the anti-inflammatory activity for many of the constructed heterocycles is extended after 24 h of inflammation induction, especially in compounds **4c,4d,4l** and **4m**.

Structure–activity relationships based on the observed results indicated that, the type of substituent attached to the phenyl group oriented at the 4'-position of the constructed heterocycles plays an important role in developing the total observed pharmacological properties. Generally, it has been found that, substituting the phenyl group with a halogen atom (either bromine, chlorine or fluorine) is associated with the decrease in the observed anti-inflammatory properties as exhibited in compounds **4a,4c,4e,4g** (potency, 125.5, 118.2, 74.7, 108.8, respectively) and **4b,4d,4f,4h** (potency, 121.8, 82.8, 80.9, 83.5, respectively). However, substitution the phenyl group with a methoxide function "as a representative example of electron-donating residue" is associated with enhancement in the observed anti-inflammatory activity as exhibited in pairs **4a,4k** (potency, 125.5, 139.3, respectively). Adoption of thienyl group "as a representative example of five-membered heterocyclic moiety" instead of the phenyl residue led to slight enhancement of the observed pharmacological properties as exhibited in pairs **4a,4l** (potency, 125.5, 126.4, respectively) and **4b,4m** (potency, 121.8, 123.5, respectively). However, no precise rule could be attained concerning the effect of methyl group attachment to the oxindolyl nitrogen on the observed anti-inflammatory properties.

3. Experimental

Melting points are uncorrected and recorded on an Electro-thermal 9100 melting point apparatus. IR spectra (KBr) were recorded on a Bruker Vector 22 spectrophotometer. ¹H NMR and ¹H,¹H-COSY spectra were recorded on a Varian MERCURY 300 (300 MHz) spectrometer. The starting compounds **1a–1g** [32–34] were prepared according to the previously reported procedures.

3.1. Synthesis of dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-diones (general procedure)

A mixture of the appropriate **1a–1g** (5 mmol), **2a,2b** (5.5 mmol) and sarcosine (5.5 mmol) in absolute ethanol (25 ml) was boiled under reflux for the appropriate time. The separated solid while refluxing was collected and crystallized from a suitable solvent affording the corresponding **4**. However, in the case of **4b,4f–4h,4j** the reaction mixture was cooled overnight (10 °C) so, the desired product was obtained.

3.1.1. 1',1''-Dimethyl-4'-phenyl-5''-(phenylmethylene)-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (**4a**)

Reaction time 24 h, colourless crystals from *n*-butanol, mp 240–242 °C (lit. 237–238 °C [35,36]), yield 86%.

Table 2

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

Compound	Mean swelling volume “ml” (% inhibition of oedema)					Potency ^c
	1 h	2 h	3 h	4 h	24 h	
Control	0.413 ± 0.065 ^b (00.0)	0.495 ± 0.098 ^b (00.0)	0.595 ± 0.128 ^b (00.0)	0.602 ± 0.066 ^b (00.0)	0.272 ± 0.063 ^b (00.0)	–
Indomethacin	0.112 ± 0.021 ^a (72.9)	0.145 ± 0.033 ^a (70.7)	0.230 ± 0.028 ^a (61.3)	0.262 ± 0.023 ^a (56.5)	0.117 ± 0.027 ^a (57.0)	100.0
4a	0.095 ± 0.028 ^a (77.0)	0.132 ± 0.033 ^a (73.3)	0.170 ± 0.037 ^a (71.4)	0.175 ± 0.052 ^{a,b} (70.9)	0.095 ± 0.041 ^a (65.1)	125.5
4b	0.132 ± 0.040 ^a (68.0)	0.145 ± 0.029 ^a (70.7)	0.145 ± 0.037 ^{a,b} (75.6)	0.188 ± 0.044 ^{a,b} (68.8)	0.200 ± 0.049 ^{a,b} (26.5)	121.8
4c	0.092 ± 0.015 ^a (77.7)	0.152 ± 0.017 ^a (69.3)	0.207 ± 0.036 ^a (65.2)	0.200 ± 0.059 ^a (66.8)	0.047 ± 0.024 ^{a,b} (82.7)	118.2
4d	0.192 ± 0.070 ^{a,b} (53.5)	0.252 ± 0.089 ^{a,b} (49.1)	0.312 ± 0.071 ^{a,b} (47.6)	0.320 ± 0.056 ^a (46.8)	0.045 ± 0.021 ^{a,b} (83.5)	82.8
4e	0.070 ± 0.017 ^{a,b} (83.1)	0.183 ± 0.087 ^a (63.0)	0.300 ± 0.108 ^{a,b} (49.6)	0.348 ± 0.118 ^{a,b} (42.2)	0.168 ± 0.063 ^{a,b} (38.2)	74.7
4f	0.073 ± 0.023 ^a (82.3)	0.187 ± 0.052 ^a (62.2)	0.330 ± 0.037 ^{a,b} (44.5)	0.327 ± 0.042 ^a (45.7)	0.138 ± 0.025 ^a (49.3)	80.9
4g	0.082 ± 0.019 ^a (80.1)	0.107 ± 0.014 ^a (78.4)	0.173 ± 0.039 ^a (70.9)	0.232 ± 0.072 ^a (61.5)	0.117 ± 0.034 ^a (57.0)	108.8
4h	0.100 ± 0.023 ^a (75.8)	0.188 ± 0.051 ^a (62.0)	0.263 ± 0.031 ^a (55.8)	0.318 ± 0.033 ^a (47.2)	0.198 ± 0.053 ^{a,b} (27.2)	83.5
4i	0.127 ± 0.044 ^a (69.2)	0.237 ± 0.060 ^{a,b} (52.1)	0.292 ± 0.042 ^a (50.9)	0.297 ± 0.073 ^a (50.7)	0.212 ± 0.064 ^{a,b} (22.1)	89.7
4j	0.192 ± 0.041 ^{a,b} (53.5)	0.188 ± 0.052 ^a (62.0)	0.208 ± 0.037 ^a (65.0)	0.188 ± 0.033 ^{a,b} (68.8)	0.117 ± 0.039 ^a (57.0)	121.8
4k	0.082 ± 0.010 ^a (80.1)	0.117 ± 0.028 ^a (76.4)	0.140 ± 0.030 ^{a,b} (76.5)	0.128 ± 0.029 ^{a,b} (78.7)	0.128 ± 0.035 ^a (52.9)	139.3
4l	0.132 ± 0.034 ^a (68.0)	0.145 ± 0.039 ^a (70.7)	0.150 ± 0.035 ^{a,b} (74.8)	0.172 ± 0.041 ^{a,b} (71.4)	0.055 ± 0.018 ^{a,b} (79.8)	126.4
4m	0.063 ± 0.015 ^{a,b} (84.7)	0.157 ± 0.044 ^a (68.3)	0.155 ± 0.042 ^{a,b} (73.9)	0.182 ± 0.040 ^{a,b} (69.8)	0.070 ± 0.031 ^a (74.3)	123.5

^a Statistically significant from the control at $p < 0.05$.^b Statistically significant from indomethacin at $p < 0.05$.^c Potency was expressed as % oedema inhibition of the tested compounds relative to % oedema inhibition of indomethacin “reference standard” at 4 h effect.

3.1.2. 4'-Phenyl-5''-(phenylmethylene)-1,1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (**4b**)

Reaction time 40 h, colourless crystals from methanol, mp 163–165 °C, yield 75%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 1706, 1685 (C=O), 1609, 1493 (C=C). ¹H NMR (CDCl₃): δ 1.64 (d, 1H, upfield H of piperidiny H₂C-2''), 2.03 (s, 3H, piperidiny NCH₃), 2.12 (s, 3H, pyrrolidiny NCH₃), 2.89 (dd, 1H, upfield H of piperidiny H₂C-6'', $J = 2.7$, 14.7 Hz), 3.13 (s, 3H, oxindolyl NCH₃), 3.28–3.36 (m, 3H, downfield H's of piperidiny H₂C-2'' and H₂C-6'' + upfield H of pyrrolidiny H₂C-5'), 4.01 (dd, 1H, downfield H of pyrrolidiny H₂C-5', $J = 8.4$, 11.1 Hz), 4.85 (dd, 1H, pyrrolidiny HC-4', $J = 6.9$, 11.1 Hz), 6.57–7.44 (m, 15H, 14 arom. H + olefinic CH). Anal. Calcd. for C₃₁H₃₁N₃O₂ (477.58): C, 77.96; H, 6.54; N, 8.80. Found: C, 78.27; H, 6.65; N, 9.02.

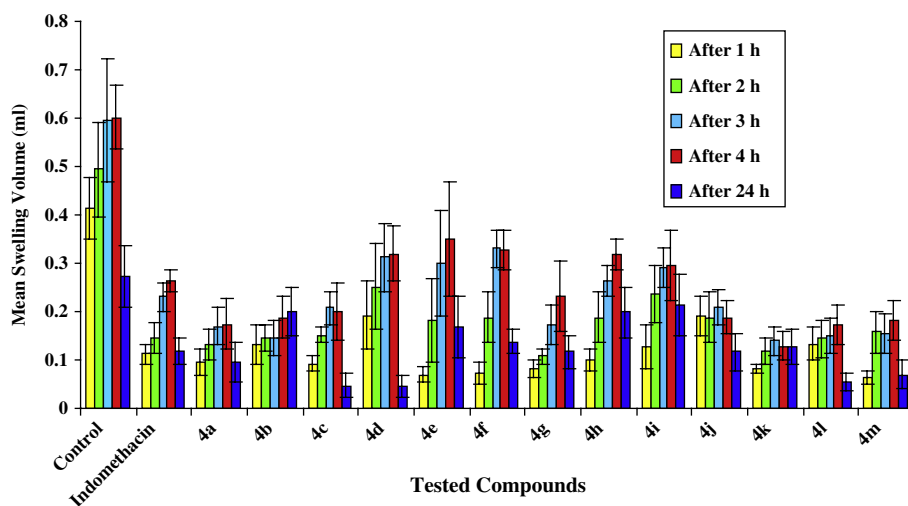
3.1.3. 4'-(4-Bromophenyl)-5''-[(4-bromophenyl)methylene]-1,1',1''-dimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (**4c**)

Reaction time 25 h, colourless crystals from *n*-butanol, mp 238–240 °C, yield 71%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3245 (NH), 1690 (br, C=O), 1609, 1486 (C=C). ¹H NMR (CDCl₃): δ 1.68 (d, 1H, upfield H of piperidiny H₂C-2'', $J = 12.9$ Hz), 2.07 (s, 3H, piperidiny NCH₃), 2.16 (s, 3H,

pyrrolidiny NCH₃), 2.90 (dd, 1H, upfield H of piperidiny H₂C-6'', $J = 2.4$, 14.7 Hz), 3.24–3.33 (m, 3H, downfield H's of piperidiny H₂C-2'' and H₂C-6'' + upfield H of pyrrolidiny H₂C-5'), 3.88 (dd, 1H, downfield H of pyrrolidiny H₂C-5', $J = 8.7$, 11.1 Hz), 4.76 (dd, 1H, pyrrolidiny HC-4', $J = 6.9$, 11.4 Hz), 6.61–7.44 (m, 13H, 12 arom. H + olefinic CH), 7.77 (s, 1H, NH). Anal. Calcd. for C₃₀H₂₇Br₂N₃O₂ (621.36): C, 57.99; H, 4.38; N, 6.76. Found: C, 58.15; H, 4.57; N, 6.50.

3.1.4. 4'-(4-Bromophenyl)-5''-[(4-bromophenyl)methylene]-1,1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (**4d**)

Reaction time 48 h, colourless crystals from *n*-butanol, mp 200–202 °C, yield 63%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 1694 (br, C=O), 1604, 1488 (C=C). ¹H NMR (CDCl₃): δ 1.62 (d, 1H, upfield H of piperidiny H₂C-2'', $J = 12.6$ Hz), 2.03 (s, 3H, piperidiny NCH₃), 2.09 (s, 3H, pyrrolidiny NCH₃), 2.85 (dd, 1H, upfield H of piperidiny H₂C-6'', $J = 2.4$, 14.7 Hz), 3.12 (s, 3H, oxindolyl NCH₃), 3.20–3.33 (m, 3H, downfield H's of piperidiny H₂C-2'' and H₂C-6'' + upfield H of pyrrolidiny H₂C-5'), 3.93 (dd, 1H, downfield H of pyrrolidiny H₂C-5', $J = 9.0$, 10.8 Hz), 4.76 (dd, 1H, pyrrolidiny HC-4', $J = 6.9$, 11.1 Hz), 6.57–7.44 (m, 13H, 12 arom. H + olefinic CH). Anal. Calcd. for C₃₁H₂₉Br₂N₃O₂ (635.38): C, 58.60; H, 4.60; N, 6.61. Found: C, 58.41; H, 4.46; N, 6.31.

**Fig. 3.** Mean oedema volume (ml) of the tested compounds at successive time intervals.

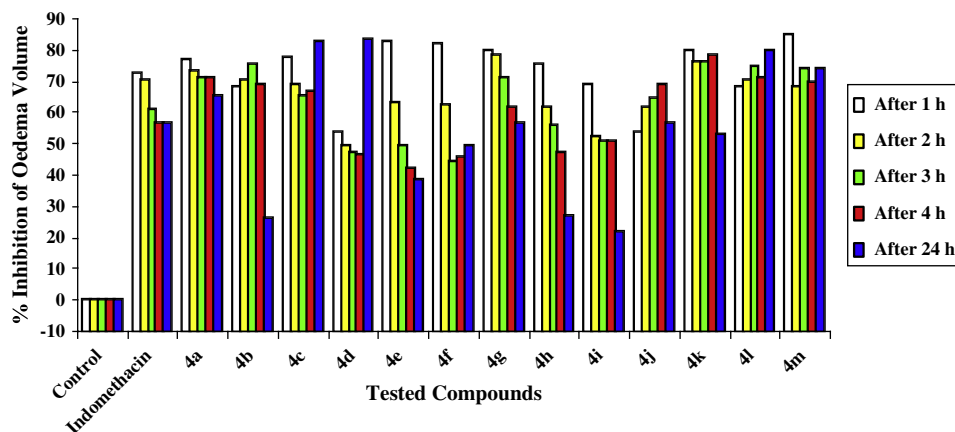


Fig. 4. % Inhibition of oedema for the tested compounds at successive time intervals.

3.1.5. 4'-(4-Chlorophenyl)-5''-[(4-chlorophenyl)methylene]-1',1''-dimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4e)

Reaction time 20 h, colourless crystals from *n*-butanol, mp 236–238 °C (lit. 238–239 °C [35]), yield 86%.

3.1.6. 4'-(4-Chlorophenyl)-5''-[(4-chlorophenyl)methylene]-1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4f)

Reaction time 42 h, colourless crystals from ethanol, mp 146–148 °C, yield 66%. IR: $\nu_{\text{max.}}/\text{cm}^{-1}$ 1688 (br, C=O), 1608, 1490 (C=C). ^1H NMR (CDCl_3): δ 1.59 (d, 1H, upfield H of piperidiny $\text{H}_2\text{C}-2''$, $J = 12.6$ Hz), 2.01 (s, 3H, piperidiny NCH_3), 2.07 (s, 3H, pyrrolidiny NCH_3), 2.83 (dd, 1H, upfield H of piperidiny $\text{H}_2\text{C}-6''$, $J = 2.7, 15.0$ Hz), 3.09 (s, 3H, oxindoly NCH_3), 3.21 (br d, 2H, downfield H's of piperidiny $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$), 3.28 (dd, 1H, upfield H of pyrrolidiny $\text{H}_2\text{C}-5'$, $J = 7.2, 8.7$ Hz), 3.91 (dd, 1H, downfield H of pyrrolidiny $\text{H}_2\text{C}-5'$, $J = 8.7, 11.1$ Hz), 4.75 (dd, 1H, pyrrolidiny $\text{HC}-4'$, $J = 6.9, 11.1$ Hz), 6.55–7.34 (m, 13H, 12 arom. H + olefinic CH). Anal. Calcd. for $\text{C}_{31}\text{H}_{29}\text{Cl}_2\text{N}_3\text{O}_2$ (546.47): C, 68.13; H, 5.35; N, 7.69. Found: C, 67.86; H, 5.24; N, 7.45.

3.1.7. 1',1''-Dimethyl-4'-(4-fluorophenyl)-5''-[(4-fluorophenyl)methylene]-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4g)

Reaction time 25 h, colourless crystals from ethanol, mp 219–221 °C, yield 72%. IR: $\nu_{\text{max.}}/\text{cm}^{-1}$ 3210 (NH), 1703, 1681 (C=O), 1610, 1508 (C=C). ^1H NMR (CDCl_3): δ 1.68 (d, 1H, upfield H of piperidiny $\text{H}_2\text{C}-2''$, $J = 12.6$ Hz), 2.07 (s, 3H, piperidiny NCH_3), 2.16

(s, 3H, pyrrolidiny NCH_3), 2.92 (dd, 1H, upfield H of piperidiny $\text{H}_2\text{C}-6''$, $J = 2.4, 14.4$ Hz), 3.24–3.35 (m, 3H, downfield H's of piperidiny $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$ + upfield H of pyrrolidiny $\text{H}_2\text{C}-5'$), 3.89 (dd, 1H, downfield H of pyrrolidiny $\text{H}_2\text{C}-5'$, $J = 8.7, 11.1$ Hz), 4.79 (dd, 1H, pyrrolidiny $\text{HC}-4'$, $J = 6.9, 11.1$ Hz), 6.61–7.39 (m, 13H, 12 arom. H + olefinic CH), 7.71 (s, 1H, NH). Anal. Calcd. for $\text{C}_{30}\text{H}_{27}\text{F}_2\text{N}_3\text{O}_2$ (499.54): C, 72.13; H, 5.45; N, 8.41. Found: C, 72.04; H, 5.30; N, 8.65.

3.1.8. 4'-(4-Fluorophenyl)-5''-[(4-fluorophenyl)methylene]-1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4h)

Reaction time 45 h, colourless crystals from methanol, mp 170–172 °C, yield 66%. IR: $\nu_{\text{max.}}/\text{cm}^{-1}$ 1685 (br, C=O), 1608, 1508 (C=C). ^1H NMR (CDCl_3): δ 1.61 (d, 1H, upfield H of piperidiny $\text{H}_2\text{C}-2''$, $J = 12.9$ Hz), 2.04 (s, 3H, piperidiny NCH_3), 2.10 (s, 3H, pyrrolidiny NCH_3), 2.86 (dd, 1H, upfield H of piperidiny $\text{H}_2\text{C}-6''$, $J = 2.4, 14.7$ Hz), 3.13 (s, 3H, oxindoly NCH_3), 3.22–3.35 (m, 3H, downfield H's of piperidiny $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$ + upfield H of pyrrolidiny $\text{H}_2\text{C}-5'$), 3.94 (dd, 1H, downfield H of pyrrolidiny $\text{H}_2\text{C}-5'$, $J = 8.7, 11.1$ Hz), 4.80 (dd, 1H, pyrrolidiny $\text{HC}-4'$, $J = 6.9, 11.1$ Hz), 6.58–7.41 (m, 13H, 12 arom. H + olefinic CH). Anal. Calcd. for $\text{C}_{31}\text{H}_{29}\text{F}_2\text{N}_3\text{O}_2$ (513.56): C, 72.50; H, 5.69; N, 8.18. Found: C, 72.34; H, 5.60; N, 8.39.

3.1.9. 1',1''-Dimethyl-4'-(4-methylphenyl)-5''-[(4-methylphenyl)methylene]-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4i)

Reaction time 30 h, colourless crystals from *n*-butanol, mp 227–229 °C (lit. 227–228 °C [35]), yield 77%.

3.1.10. 4'-(4-Methylphenyl)-5''-[(4-methylphenyl)methylene]-1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4j)

Reaction time 50 h, colourless crystals from methanol, mp 186–188 °C, yield 71%. IR: $\nu_{\text{max.}}/\text{cm}^{-1}$ 1696 (br, C=O), 1598, 1512 (C=C). ^1H NMR (CDCl_3): δ 1.66 (d, 1H, upfield H of piperidiny $\text{H}_2\text{C}-2''$, $J = 12.9$ Hz), 2.04 (s, 3H, piperidiny NCH_3), 2.11 (s, 3H, pyrrolidiny NCH_3), 2.32 (s, 3H, ArCH_3), 2.34 (s, 3H, ArCH_3), 2.89 (dd, 1H, upfield H of piperidiny $\text{H}_2\text{C}-6''$, $J = 2.4, 15.0$ Hz), 3.12 (s, 3H, oxindoly NCH_3), 3.27–3.33 (m, 3H, downfield H's of piperidiny $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$ + upfield H of pyrrolidiny $\text{H}_2\text{C}-5'$), 3.98 (dd, 1H, downfield H of pyrrolidiny $\text{H}_2\text{C}-5'$, $J = 8.7, 11.4$ Hz), 4.81 (dd, 1H, pyrrolidiny $\text{HC}-4'$, $J = 6.9, 11.4$ Hz), 6.56–7.32 (m, 13H, 12 arom. H + olefinic CH). Anal. Calcd. for $\text{C}_{33}\text{H}_{35}\text{N}_3\text{O}_2$ (505.63): C, 78.38; H, 6.98; N, 8.31. Found: C, 78.67; H, 7.28; N, 8.47.

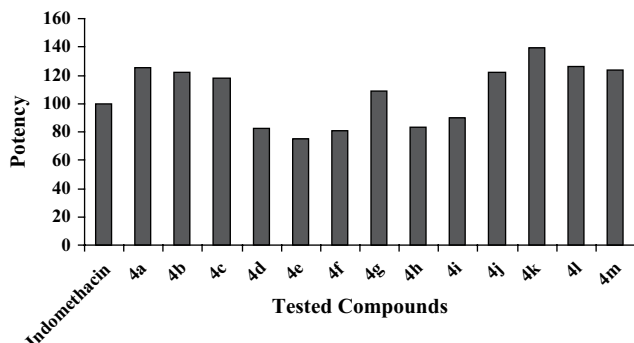


Fig. 5. Anti-inflammatory activity potency of the tested compounds relative to indomethacin which was used as a reference standard.

3.1.11. 1',1''-Dimethyl-4'-(4-methoxyphenyl)-5''-[(4-methoxyphenyl)methylene]-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4k**)**

Reaction time 30 h, pale yellow crystals from *n*-butanol, mp 216–218 °C (lit. 213–214 °C [35]), yield 65%.

3.1.12. 1',1''-Dimethyl-4'-(2-thienyl)-5''-[(2-thienyl)methylene]-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4l**)**

Reaction time 30 h, pale yellow crystals from *N,N*-dimethylformamide–water mixture as 3:2 v/v, mp 248–250 °C, yield 88%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3195 (NH), 1691 (br, C=O), 1613, 1577 (C=C). ^1H NMR (DMSO- d_6): δ 1.82 (d, 1H, upfield H of piperidinyl $\text{H}_2\text{C}-2''$, $J = 10.8$ Hz), 1.95 (s, 3H, piperidinyl NCH_3), 2.07 (s, 3H, pyrrolidinyl NCH_3), 2.96 (dd, 1H, upfield H of piperidinyl $\text{H}_2\text{C}-6''$, $J = 2.4$, 15.9 Hz), 3.23–3.35 (m, 3H, downfield H's of piperidinyl $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$ + upfield H of pyrrolidinyl $\text{H}_2\text{C}-5'$), 3.75 (t, 1H, downfield H of pyrrolidinyl $\text{H}_2\text{C}-5'$, $J = 9.0$ Hz), 4.85 (t, 1H, pyrrolidinyl $\text{HC}-4'$, $J = 9.3$ Hz), 6.59–7.79 (m, 11H, 10 arom. H + olefinic CH), 10.31 (s, 1H, NH). Anal. Calcd. for $\text{C}_{26}\text{H}_{25}\text{N}_3\text{O}_2\text{S}_2$ (475.60): C, 65.66; H, 5.30; N, 8.84. Found: C, 65.43; H, 5.24; N, 8.67.

3.1.13. 4'-(2-Thienyl)-5''-[(2-thienyl)methylene]-1,1',1''-trimethyl-dispiro[3H-indole-3,2'-pyrrolidine-3',3''-piperidine]-2(1H),4''-dione (4m**)**

Reaction time 48 h, pale yellow crystals from ethanol, mp 193–195 °C, yield 78%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 1697, 1674 (C=O), 1609, 1579 (C=C). ^1H NMR (CDCl_3): δ 1.91 (d, 1H, upfield H of piperidinyl $\text{H}_2\text{C}-2''$, $J = 12.9$ Hz), 2.09 (s, 3H, piperidinyl NCH_3), 2.12 (s, 3H, pyrrolidinyl NCH_3), 2.96 (dd, 1H, upfield H of piperidinyl $\text{H}_2\text{C}-6''$, $J = 2.4$, 15.9 Hz), 3.18 (s, 3H, oxindolyl NCH_3), 3.32 (br d, 2H downfield H's of piperidinyl $\text{H}_2\text{C}-2''$ and $\text{H}_2\text{C}-6''$), 3.45 (dd, 1H, upfield H of pyrrolidinyl $\text{H}_2\text{C}-5'$, $J = 7.5$, 9.0 Hz), 3.95 (dd, 1H, downfield H of pyrrolidinyl $\text{H}_2\text{C}-5'$, $J = 8.7$, 10.8 Hz), 5.06 (dd, 1H, pyrrolidinyl $\text{HC}-4'$, $J = 7.2$, 10.8 Hz), 6.60–7.47 (m, 10H, arom. H), 7.57 (br s, 1H, olefinic CH). Anal. Calcd. for $\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_2\text{S}_2$ (489.63): C, 66.23; H, 5.56; N, 8.58. Found: C, 66.04; H, 5.38; N, 8.49.

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

For X-ray crystallographic studies, compound **4k** was recrystallized as prismatic pale yellow crystals from *n*-butanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo- $\text{K}\alpha$ radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR92 [37] and refined by maXus [38] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $\text{C}_{26}\text{H}_{25}\text{N}_3\text{O}_4$, $M_r = 523.633$, triclinic, crystallizes in space group P_1 , cell lengths " $a = 11.6129(3)$ ", " $b = 11.7112(3)$ ", " $c = 11.8220(4)$ Å", cell angles " $\alpha = 97.2182(12)$ ", " $\beta = 94.4209(14)$ ", " $\gamma = 117.0740(10)^\circ$ ", $V = 1403.77(7)$ Å³, $Z = 2$, $D_c = 1.239$ mg/m³, θ values 2.910–24.713°, absorption coefficient μ (Mo- $\text{K}\alpha$) = 0.08 mm^{−1}, $F(000) = 556$. The unique reflections measured 5303 of which 2874 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 352 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000 F_o^2]$. The final agreement factors were $R = 0.047$ and $wR = 0.089$ with a goodness-of-fit of 1.352. Full crystallographic details, excluding structure factors have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 695778.

3.3. Anti-tumor activity screening

Anti-tumor activity screening for the synthesized compounds (**4e**, **4f**, **4k**, **4m**) at a dose of 10 μM utilizing 59 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 [24–27]. 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 the 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 $\mu\text{g}/\text{ml}$ 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 read 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 [28–31]. 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 at a dose of 10 mg/kg body weight) and the tested compounds (**4a**–**4m**) dissolved in DMSO, at 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, 4 and 24 h) with Plethysmometer 7140 (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 \pm 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. 3–5).

$$\% \text{ Inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

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.

$$\text{Potency} = (\% \text{ Oedema inhibition of tested compound treated group}) / (\% \text{ Oedema inhibition of indomethacin treated group})$$

Acknowledgment

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