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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, bioactivity, and POM analyses of isothiochromeno[3,4-*e*][1,2]oxazines

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Abstract A series of 18 new 3,4-disubstituted-isothiochromeno[3,4-*e*][1,2]oxazines **28–45** has been obtained from the 3′,4′-di-substituted-4′H-spiro[isothiochromene-3,5′-isoxazol]-4(1H)-ones **10–27** in refluxing HCl acid/ethanol. A series of 15/18 compounds **28–45** was selected by the National Cancer Institute (NCI, Bethesda, USA) and were evaluated against a full panel of 60 primary human tumor cell lines derived from nine human cancer types, all of which showed antiproliferative activity in the micromolar range. The most active compound number **37** (S722910) showed high potency against all the tested cell lines with a GI_{50} mean value in the range of 30–80 μM; TGI and LC_{50} values were 12–16 μM having positive response on 98 and 63 % of the tested cell lines (Breast-MCF7 and NCS-SF-268) respectively.

Keywords Leukemia · Breast cancer inhibition · Spiro-isothiochromeno[3,4-*e*][1,2]oxazines · POM analyses

Abbreviation

GI_{50}	Growth inhibition; the drug concentration resulting in a 50 % reduction in the net protein increase compared with control cells during the drug incubation
TGI	Cytostatic activity; the drug concentration resulting in total growth inhibition
LC_{50}	\The concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment compared with that at the beginning, thus indicating a net loss of cells following treatment
POM	Petra/Osiris/Molinspiration

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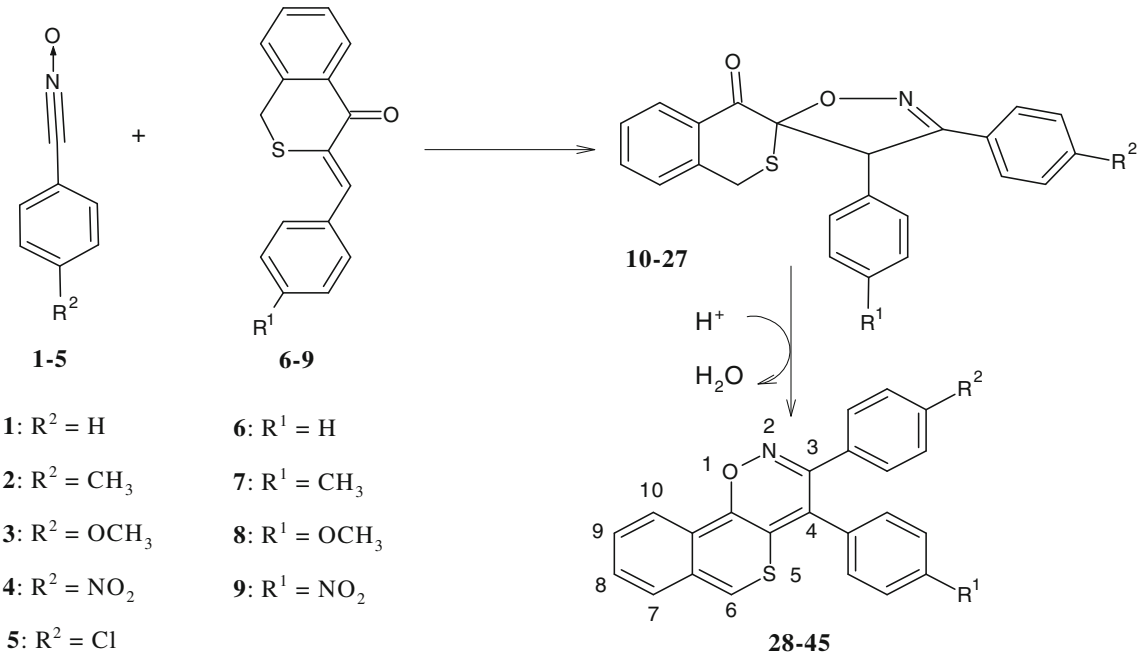
Introduction

Isoxazoline derivatives have been shown to be efficient precursors for many synthetic intermediates including γ-amino alcohols and β-hydroxy ketones (Kozikowski, 1984; Kanemasa *et al.*, 1990). Spiro-isoxazolines display interesting biologic properties such as herbicidal, plant-growth regulatory, and antitumor (Howe and Shelton, 1990; Smietana *et al.*, 1999). Many 4-chromanone derivatives are versatile intermediates for the synthesis of natural products such as brazillin, hematoxylin, ripariochromene, clausenin, calonlide A, and inophyllum (Ellis *et al.*, 1997; Chenera *et al.*, 1993). Chromanone heterocycles have attracted much attention owing to their important pharmacological properties (Ellis *et al.*, 1997). Their high synthetic utility and pharmacological importance have prompted us to synthesize and study the antitumor activity of some previously prepared spiro-isoxazolidine compounds **10–27** and their new aromatic planar derivatives **28–45** (Scheme 1).

Currently, we are working on the synthesis and pharmacomodulation of spiro-heterocyclic compounds, which have dual antibacterial/antiviral pharmacophores, such as spiro-isoxazolines (Bennani *et al.*, 2007a; 2004; Al Houari *et al.*, 2008), spiro-isoquinolines (Hadda *et al.*, 2007, 2008; Akkurt *et al.*, 2006, 2010), and spiro-isoxazolidin (Bennani *et al.*, 2007b). This will allow us to gain insight into the therapeutic behavior of larger heterocyclic systems containing both isothiochromane and isoxazol rings as potential therapeutic agents, and delocalised π-conjugated rigid pharmacophore site(s). The biologic activities of these compounds and their derivatives were determined by High Screening Techniques (Gray and Wickstrom, 1996) to investigate their suitability as a new class of antitumor agents.



1: $R^2 = H$ **6**: $R^1 = H$

2: $R^2 = CH_3$ **7**: $R^1 = CH_3$

3: $R^2 = OCH_3$ **8**: $R^1 = OCH_3$

4: $R^2 = NO_2$ **9**: $R^1 = NO_2$

5: $R^2 = Cl$

10, **28**: $(R^1, R^2) = (H, H)$ **16**, **34**: $(R^1, R^2) = (CH_3, CH_3)$ **22**, **40**: $(R^1, R^2) = (OCH_3, OCH_3)$

11, **29**: $(R^1, R^2) = (H, CH_3)$ **17**, **35**: $(R^1, R^2) = (CH_3, OCH_3)$ **23**, **41**: $(R^1, R^2) = (OCH_3, NO_2)$

12, **30**: $(R^1, R^2) = (H, OCH_3)$ **18**, **36**: $(R^1, R^2) = (CH_3, NO_2)$ **24**, **42**: $(R^1, R^2) = (OCH_3, Cl)$

13, **31**: $(R^1, R^2) = (H, NO_2)$ **19**, **37**: $(R^1, R^2) = (CH_3, Cl)$ **25**, **43**: $(R^1, R^2) = (NO_2, CH_3)$

14, **32**: $(R^1, R^2) = (H, Cl)$ **20**, **38**: $(R^1, R^2) = (OCH_3, H)$ **26**, **44**: $(R^1, R^2) = (NO_2, OCH_3)$

15, **33**: $(R^1, R^2) = (CH_3, H)$ **21**, **39**: $(R^1, R^2) = (OCH_3, CH_3)$ **27**, **45**: $(R^1, R^2) = (NO_2, Cl)$

Scheme 1 Synthesis of new compounds **28–45** from precursors **10–27**

Table 1 ^{1}H NMR data (δ in ppm; *J* in Hz) of selected compounds of series **28–45**

Compound	(R_1, R_2)	δR_1	δR_2	δH^6	δH^9	δH (Arom)
28	(H, H)	–	–	7.70	8.30	7.10–7.60
				(s, 1H)	(dd, $J_o = 9$; $J_m = 2$)	(m, 13H)
33	(CH_3, H)	2.50	–	7.70	8.35	7.15–7.60
		(s, 3H)		(s, 1H)	(dd, $J_o = 9.0$; $J_m = 1.0$)	(m, 12H)
34	(CH_3, CH_3)	2.40	2.50	7.70	8.30	7.10–7.60
		(s, 3H)	(s, 3H)	(s, 1H)	(dd, $J_o = 9.0$; $J_m = 1.0$)	(m, 11H)
35	(CH_3, OCH_3)	2.45	3.80	7.7	8.30	6.80–7.60
		(s, 3H)	(s, 3H)	(s, 1H)	(dd, $J_o = 9.0$, $J_m = 1.0$))	(m, 11H)
36	(CH_3, NO_2)	2.50	–	7.75	8.30	7.15–8.20
		(s, 3H)		(s, 1H)	(dd, $J_o = 9.0$; $J_m = 1.0$)	(m, 11H)
45	(NO_2, Cl)	–	–	7.80	8.10	7.15–8.30
				(s, 1H)	(dd, $J_o = 9.0$; $J_m = 1.0$)	(m, 11H)

Table 2 ^{13}C NMR data (δ in ppm) of selected compounds of series **28–45**

Compounds	(R_1,R_2)	δ R_1	δ R_2	δC^6	δC^3	δC^{10b}	δ C–H aromat.	δ C aromat.
28	(H, H)	–	–	120.80	161.50	163.00	120.10; 122.20; 124.30; 125.95; 128.30; 128.50; 128.90; 129.30; 129.50; 131.00	114.80; 118.40; 128.70; 130.10; 136.70; 138.60
33	(CH_3, H)	21.50	–	120.80	161.60	163.00	122.20; 122.50; 124.50; 126.00; 128.40; 128.50; 129.50; 130.10; 130.90	115.00; 118.70; 127.00; 129.05; 136.70; 138.60; 140.80
34	(CH_3, CH_3)	21.40	21.50	120.60	161.50	162.80	122.10; 122.40; 124.30; 125.90; 128.20; 129.25; 130.05; 130.90	114.80; 118.80; 126.10; 127.15; 136.70; 138.55; 138.60; 139.50
35	(CH_3, OCH_3)	21.50	55.20	120.60	161.10	162.80	114.00; 122.12; 122.40; 124.30; 125.90; 128.70 130.20; 131.00	114.70; 118.80; 121.40; 127.25; 136.70; 138.60 160.60
36	(CH_3, NO_2)	21.50	–	121.45	159.70	163.90	122.15; 122.20; 123.70; 124.50; 126.30; 129.10; 130.50; 130.70	114.55; 117.95; 126.15;135.40; 136.90; 138.60; 139.30; 148.30
45	(NO_2, Cl)	–	–	121.80	160.60	163.60	121.90; 122.40; 124.50; 124.70; 126.70; 129.20; 129.70; 131.90	112.80; 116.85; 124.50; 136.25; 137.00; 137.20; 138.90; 147.90

Results and discussion

Synthesis

The starting materials, 3′,4′-di-substituted-4′H-spiro[isothiochromene-3,5′-isoxazol]-4(1H)-ones **10–27**, were obtained in good yield from the corresponding *p*-substituted benzaldoximes **1–5** by 1,3-dipolar cycloaddition with appropriate (3Z)-3-(4-methylbenzylidene)-1H-isothiochromen-4(3H)-ones **6–9** by our previously reported procedure (Bennani *et al.*, 2007a; 2004; Al Houari *et al.*, 2008). The new compounds **28–45** were prepared in good yields by acidic hydrolysis of precursors **10–27** with one equivalent of HCl in reluxing ethanol (Scheme 1).

Compounds **10–27** were previously prepared and characterized in our laboratory (Bennani *et al.*, 2007a; 2004; Al Houari *et al.*, 2008). The new compounds **28–45** were stable at ambient temperature. Their structures were determined by IR, MS, and NMR spectroscopy. The selected NMR data are regrouped in Tables 1 and 2.

Evaluation of in vitro antitumoral activity of **28–45**

A series of disubstituted isothiochromeno[3,4-*e*][1,2]oxazines **28–45** were selected by the National Cancer Institute for evaluation of their in vitro anticancer activity (Table 3).

As shown in Table 3, compound **37** is the only derivative that shows cytotoxic activity in this series. Lipophilicity properties can be evaluated by experimental (shake-flask method) or fragmentation methods. However, neither the degree of lipophilicity (LogP) nor the molecular polar surface area (TSPA) correlates positively with the cytotoxic activity. Compounds **34** and **37** were further tested for their in vitro activity against 60 primary human cancer cell lines (Tables 4, 5, 6, 7).

From the data in Table 3, it appeared that only the isothiochromeno-oxazines **34** and **37** showed marked cytotoxic activity compared to the rest of series **28–45**. In this case, the degree of lipophilicity of the aryls substituent does not correlate positively with the cytotoxic activity. A direct influence of the lipophilic group from the

Table 3 In vitro cytotoxic activity of selected compounds against three cell lines

Compounds	(NCI-Ref.)	(R_1, R_2)	Growth percentages			Activity
			(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268	
28	(S722904)	(H, H)	94	104	97	Inactive
29	(S722914)	(H, Me)	92	51	66	Inactive
30	(S722917)	(H, OMe)	101	97	106	Inactive
31	(S722918)	(H, NO_2)	100	62	101	Inactive
32	(S722915)	(H, Cl)	92	95	95	Inactive
33	(S722916)	(Me, H)	94	41	69	Inactive
34	(S722913)	(Me, Me)	93	36	91	Active
35	(S722907)	(Me, OMe)	96	86	97	Inactive
36	(S722908)	(Me, NO_2)	99	97	83	Inactive
37	(S722910)	(Me, Cl)	91	2	37	Active
39	(S722905)	(OMe, Me)	84	98	103	Inactive
40	(S722909)	(OMe, OMe)	102	116	105	Inactive
42	(S722906)	(OMe, Cl)	85	51	102	Inactive
43	(S722912)	(NO_2, Me)	97	99	111	Inactive
45	(S722911)	(NO_2, Cl)	97	85	104	Inactive

Table 4 Comparative study of in vitro antitumor activity of hits **34** and **37** against leukemia and non-small cell lung cancer cell lines

Panel	Cell line	GI_{50} (μM) for **34** & **37**	
		34	**37**
Leukemia	HL-60(TB)	1.00×10^{-4}	1.82×10^{-5}
	K-562	2.29×10^{-5}	2.06×10^{-5}
	MOLT-4	3.71×10^{-5}	1.25×10^{-5}
	RPMI-8226	6.92×10^{-5}	1.18×10^{-5}
	SR	4.20×10^{-5}	3.65×10^{-5}
Non-small cell lung	A549/ATCC	$>10^{-4}$	$>10^{-4}$
	EKVX	$>10^{-4}$	$>10^{-4}$
	HOP-62	$>10^{-4}$	$>10^{-4}$
	HOP-92	6.81×10^{-5}	2.65×10^{-5}
	NCI-H226	$>10^{-4}$	$>10^{-4}$
	NCI-H23	$>10^{-4}$	1.66×10^{-5}
	NCI-H322M	$>10^{-4}$	$>10^{-4}$
	NCI-H460	$>10^{-4}$	$>10^{-4}$
	NCI-H522	5.38×10^{-5}	1.51×10^{-5}

isothiochromeno nucleus appeared to be more important for activity. The in vitro antitumor activity of compounds **34** and **37** were further investigated (Tables 4, 5, 6).

A comparison of antitumor screening results of compounds **34** and **37** with regard to their in vitro activity against 60 cell lines reveals mechanistic specificity notably in the leukemia and breast cancer cell lines (Tables 5, 6). Compound **37** has micromolar activity against all the leukemia cell lines, while its other analogs have no activity against one of the same strains. It appears that in this limited series, the presence of a more polar substituent on the oxazole ring enhances the activity. Further enhancement of activity was attempted, but not achieved by the substitution of the second aryl group for compound **40** on the para position (compound **40** vs. **37**). There appears to be no gain in activity with the disubstituted-analogs compared to the corresponding *mono*-substituted compound **37** (data not shown).

Table 5 Comparative in vitro antitumor activity of diastereoisomers **34** and **37** against CNS, melanoma, ovarian, and renal cancer cell lines

Panel	Cell line	GI_{50} (μM) for **34** & **37**	
		34	**37**
CNS cancer	SF-268	$>10^{-4}$	1.21×10^{-5}
	SF-295	$>10^{-4}$	$>10^{-4}$
	SF-539	$>10^{-4}$	$>10^{-4}$
	SNB-19	$>10^{-4}$	$>10^{-4}$
	U251	$>10^{-4}$	2×10^{-5}
Melanoma	LOX IMVI	$>10^{-4}$	5×10^{-5}
	MALME-3M	$>10^{-4}$	2.21×10^{-5}
	M14	$>10^{-4}$	$>10^{-4}$
	SK-MEL-2	$>10^{-4}$	$>10^{-4}$
	SK-MEL-28	$>10^{-4}$	$>10^{-4}$
	SK-MEL-5	$>10^{-4}$	5.31×10^{-5}
	UACC-257	$>10^{-4}$	2.56×10^{-5}
	UACC-62	$>10^{-4}$	$>10^{-4}$
Ovarian cancer	IGROV1	$>10^{-4}$	10^{-5}
	OVCAR-3	9.48×10^{-5}	$> \times 10^{-4}$
	OVCAR-4	$>10^{-4}$	$>10^{-4}$
	OVCAR-5	$>10^{-4}$	$>10^{-4}$
	OVCAR-8	4×10^{-5}	1.16×10^{-5}
Renal cancer	786-0	$>10^{-4}$	2.12×10^{-5}
	A498	$>10^{-4}$	$>10^{-4}$
	ACHIN	$>10^{-4}$	$>10^{-4}$
	CAKI-1	$>10^{-4}$	$>10^{-4}$
	RXF 393	$>10^{-4}$	$>10^{-4}$
	SN12C	2×10^{-5}	1.13×10^{-5}
	TK-10	$>10^{-4}$	$>10^{-4}$
	UO-31	$>10^{-4}$	$>10^{-4}$

Table 6 Comparative in vitro antitumor activity of hits **34** and **37** against prostate, breast, and colon cancer cell lines

Panel	Cell line	GI_{50} (μM) for **34** & **37**	
		34	**37**
	PC-3	$>10^{-4}$	2.16×10^{-5}
Prostate cancer	DU-145	$>10^{-4}$	$>10^{-4}$
Breast cancer	MCF7	$>10^{-4}$	3.12×10^{-5}
	NCI/ADR-RES	$>10^{-4}$	$>10^{-4}$
	MDA-MB-231/ATCC	$>10^{-4}$	$>10^{-4}$
	HS 578T	$>10^{-4}$	$>10^{-4}$
	MDA-MB-435	$>10^{-4}$	4.42×10^{-5}
	BT-549	$>10^{-4}$	4.12×10^{-5}
	T-47D	2.54×10^{-5}	3.14×10^{-5}
Colon cancer	COLO 205	$>10^{-4}$	4.62×10^{-5}
	HCC-2998	$>10^{-4}$	$>10^{-4}$
	HCT-116	5.75×10^{-5}	6.27×10^{-5}
	HCT-15	$>10^{-4}$	8.12×10^{-5}
	HT29	$>10^{-4}$	$>10^{-4}$
	KM12	$>10^{-4}$	$>10^{-4}$
	SW-620	$>10^{-4}$	5.56×10^{-5}

Table 7 Osiris calculations of compounds **28–45**

Compd.	MW	Toxicity risks[a]				Osiris calculations[b]			
		MUT	TUMO	IRRI	REP	CLP	S[a]	DL[a]	D-S[a]
28	353	–	–	–	+	6.13	−5.62	−0.09	0.23
29	367	–	–	–	+	6.45	−5.97	2.00	0.26
30	383	–	–	–	+	6.03	−5.54	3.54	0.30
31	398	–	–	–	+	6.00	−6.08	−6.71	0.14
32	387	–	–	–	+	6.75	−6.36	4.66	0.25
33	367	–	–	–	–	6.45	−5.97	2.46	0.33
34	381	–	–	–	–	6.76	−5.31	1.88	0.29
35	397	–	–	–	–	6.34	−5.98	2.15	0.32
36	412	–	–	–	–	6.32	−6.43	−8.10	0.16
37	401	–	–	–	–	7.06	−6.70	3.27	0.29
38	383	–	–	–	–	6.03	−5.54	3.74	0.38
39	397	–	–	–	–	6.34	−5.98	1.89	0.32
40	413	–	–	–	–	5.92	−5.56	3.16	0.37
41	428	–	–	–	–	5.90	−5.10	−5.81	0.17
42	417	–	–	–	–	6.64	−6.38	4.54	0.30
43	412	–	–	–	–	6.32	−5.43	−8.36	0.16
44	428	–	–	–	–	5.90	−6.10	−5.81	0.17
45	432	–	–	–	–	6.62	−6.82	−5.68	0.14
Campto[c]	350	+	+	–	+	1.18	−3.04	5.24	0.25

Active risk = −, non-active risk = +

[a] *MUT* mutagenic, *TUMO* tumorogenic, *IRRI* irritant, *REP* reproductive effective

[b] *CLP* cLogP, *S* solubility, *DL* druglikness, *D-S* drug-score

[c] *Campto* camptothecin

POM molecular virtual screening

Osiris calculations

Structure-based design is now fairly routine, but many potential drugs fail to reach the clinic because of ADME-Tox liabilities. One very important class of enzymes responsible for many ADMET problems is the cytochromes P450 (Hokuldsson, 1988). Inhibition of these or the production of unwanted metabolites can result in many adverse drug reactions. Of the most important program, Osiris is already available online and it may be used in success with complementary Molinspiration and Petra software (POM) as we have previously demonstrated (Sheikh *et al.*, 2011; Chohan *et al.*, 2010). Prediction results of compounds **28–45** toxicity risks (MUT, TUMO, IRRI, and REP) are valued (Table 7). It appears that the new series **28–45** is less toxic than clinical drug, the camptothecin (campto). This is a great advantage in favor of the titled candidates **28–45**. On other hand, CLP constitutes a serious problem (CLP should be inferior to 5). This means there are more phenyl than it should be. The D-S of compounds **28–45** is encouraging (D-S = 0.14–0.38); it is similar or more important than D-S of Compto (DS = 0.25).

Molinspiration calculations

Drug likeness is calculated by the methodology developed by Molinspiration (Table 8). The method is very robust and is able to process practically all organic and most organometallic molecules. Molecular Polar Surface Area TPSA is calculated based on the methodology published (Ertl *et al.*, 2000) as a sum of fragment contributions. O– and N– centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. Prediction results of compounds **28–45** molecular properties (TPSA, GPCR ligand, and ICM) are valued (Tables 7, 8, 9).

Pi-charge calculations

PETRA is a software package comprising various empirical methods for the calculation of physicochemical properties in organic molecules. All 18 methods are empirical in nature. The following chemical effects can be quantified: heats of formation, bond dissociation energies, sigma charge distribution, π-charge distribution, inductive effect, resonance effect and delocalization energies, and polarizability effect.

Table 8 Molinspiration calculations of compounds **28–45**

Compd	Molinspiration calculations[a]				Drug-likeness[b]					
	TPSA	NONI	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
28	26	0	1	310	−0.02	−0.14	0.08	0.06	−0.17	0.05
29	26	0	1	327	−0.06	−0.20	0.03	0.03	−0.22	−0.02
30	35	0	1	336	−0.07	−0.20	0.04	0.04	−0.22	−0.01
31	72	0	1	334	−0.16	−0.18	−0.06	−0.04	−0.29	−0.07
32	26	0	1	324	−0.02	−0.14	0.06	0.04	−0.21	0.01
33	26	0	1	333	0.13	0.03	0.11	0.18	−0.01	0.14
34	22	0	1	350	−0.24	−0.39	−0.36	−0.09	−0.21	−0.15
35	35	0	1	352	−0.10	−0.26	−0.01	0.01	−0.26	−0.06
36	72	0	1	350	−0.19	−0.24	−0.10	−0.07	−0.33	−0.12
37	26	0	1	340	−0.06	−0.20	0.01	0.01	−0.26	−0.05
38	35	0	1	336	−0.07	−0.20	0.04	0.04	−0.22	−0.01
39	35	0	1	352	−0.10	−0.26	−0.01	0.01	−0.26	−0.06
40	45	0	1	361	−0.06	−0.19	0.03	0.04	−0.20	−0.01
41	72	0	1	350	−0.19	−0.24	−0.10	−0.07	−0.33	−0.12
42	35	0	1	349	−0.07	−0.20	0.02	0.02	−0.25	−0.04
43	72	0	1	350	−0.19	−0.24	−0.10	−0.07	−0.33	−0.12
44	81	0	1	359	−0.19	−0.23	−0.09	−0.06	−0.32	−0.11
45	72	0	1	347	−0.16	−0.18	−0.07	−0.06	−0.32	−0.10

[a] *NONI* number OH–NH interaction, *NV* Number of violation, *VOL* volume

[b] *GPCR* GPCR ligand, *ICM* ion channel modulator, *KI* kinas inhibitor, *PI* protease inhibitor, *EI* enzyme inhibitor

Table 9 Selected π-charge of heteroatoms of compounds **28–45**

Compound	Substituents		Partial π-charge of heteroatom (in e)		
	R_1	R_2	O_1	N_2	S_5
28	H	H	−0.09	−0.00	−0.15
29	H	CH_3	−0.09	−0.00	−0.15
30	H	OCH_3	−0.07	−0.01	−0.12
31	H	NO_2	−0.09	−0.01	−0.10
32	H	Cl	−0.09	−0.00	−0.15
33	CH_3	H	−0.09	−0.00	−0.15
34	CH_3	CH_3	−0.09	−0.00	−0.15
35	CH_3	OCH_3	−0.07	−0.01	−0.12
36	CH_3	NO_2	−0.09	−0.01	−0.10
37	CH_3	Cl	−0.09	−0.00	−0.15
38	OCH_3	H	−0.05	−0.01	−0.17
39	OCH_3	CH_3	−0.05	−0.01	−0.11
40	OCH_3	OCH_3	−0.04	−0.02	−0.09
41	OCH_3	NO_2	−0.06	−0.02	−0.08
42	OCH_3	Cl	−0.05	−0.01	−0.11
43	NO_2	CH_3	−0.04	−0.02	−0.13
44	NO_2	OCH_3	−0.03	−0.02	−0.12
45	NO_2	Cl	−0.04	−0.02	−0.13

The series **28–45** of oxazolidines have been subjected to delocalized-charge calculations by the PETRA method of the nonhydrogen common atoms, obtained from the partial π-charge of the heteroatoms, have been used to model the bioactivity against cancer. We give here, as example, the compounds **36** and **37**. It is found that the negative charges

of the oxygen and nitrogen atoms of oxazolidine group and the partial π positive charges of sulfur and supplementary aryls contribute positively in favor of an antitumoral activity, and this is in good agreement with the mode of antitumoral action of the compounds bearing ($X^{\delta-}$–Spacer–$Y^{\delta+}$) pharmacophore(s) site. It was hypothesized that difference in charges between two heteroatoms of the same pharmacophore site ($X^{\delta-}$ spacer $Y^{\delta+}$) may facilitate the intercalation in DNA of cancerous strains. It is further found that the activity increases with increase in negative charge of one heteroatom of the common pharmacophore fragment of the compounds (**36** and **37**). This possible synergistic and streamlined working procedure may lead to highly active isomeric/tautomeric DNA receptor ligands. In fact, the dissymmetric isoxazoline 37, which can apparently recognize specific DNA sequences, could have potential for gene targeting which merits investigation of its capacity to inhibit access of activators or repressors to regulatory sites in DNA controlling gene expression (Waring *et al.*, 2002).

Conclusion

Hydrolysis of spiro-compounds **10–27** with hydrochloric acid in the refluxing ethanol has been demonstrated to be a powerful and optimal method for isothiochromane-oxazole aromatic cyclization in the preparation of a new heterocyclic family, the 3,4-di-substituted-isothiochromeno[3,4-*e*][1,2]oxazines **28–45**. This work provides for the first time a simple one-pot synthetic methodology for the preparation of a wide range of **28–45** compounds which have analog or hybrid *O,S*-pharmacophore site of *O,O*-pharmacophore site of the natural alkaloid Camptothecin (Campto). The cytotoxic activity of this new family suggests a promising novel approach to the design of prospective compounds for the treatment of Leukemia and breast cancer.

As a guide for future work, the data reported here indicate that the **28–45** compounds have a definite potential efficacy that merits development through modification to both the isothiochromeno and the substituents on C-2 and C-3 of oxazine ring. We note, however, that although the compounds are not nominally camptothecin analogs, there is no necessary presumption that they do in fact resemble camptothecin and thereby target topoisomerase-I. It might be possible to address this question by testing their effect(s) on two cell lines, one of which has normal topoisomerase I and the other has a mutant (camptothecin-resistant) enzyme. If there was a difference in GI_{50} value, this would indicate that topoisomerase I is a critical target for the drugs. Given the rather low GI_{50} values of the present compounds, around 200 μM, it would be prudent first to seek new derivatives having GI_{50} values around 1 micromolar or less so that the assay can be conducted more efficiently, though the interest in compounds **34** and **37** due to their particular stereochemistry remains undiminished. A further consideration relates to the poor solubility of the aromatic derivatives in water; for the cell line assays to work, it is important to prepare derivatives that are more soluble. Substituting the two aryls on C2 and C3 of oxazine ring with some other groups such as alkyloxy or amino is possible and might produce the desired effect.

Experimental protocols

General procedure

NMR spectra (^1H, ^{13}C) were recorded on a Bruker DPX 200 (operating at 200.12 MHz for ^1H, 50.32 MHz for ^{13}C) or on a Bruker AM 300 (operating at 300.13 MHz for ^1H, at 75.47 MHz for ^{13}C) spectrometer. NMR data are listed in ppm and are reported relative to tetramethylsilane (^1H, ^{13}C), residual solvent peaks being used as internal standard with external calibration. Complete assignments of the ^{13}C spectra required non-decoupled ^{13}C NMR spectra with selective. Infra-red spectra were recorded in KBr pellets using a Brucker IFS28 FTIR spectrometer. Mass spectrometer (70 eV) and elemental analysis were performed by the Service du Centre Universitaire Régional d'Interface CURI (University Sidi Mohamed Ben Abdellah, Fès, Morocco).

Biologic evaluation

In vitro anticancer activity was measured by the National Cancer Institute (NCI/USA) against three cell lines, NCI H460 (Lung), MCF7 (Breast), and SF-268 (CNS) as a preliminary step. Then, the obtained hits are tested against 60 tumor types. In the current protocol, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for 48 h. End-point determinations were made using Alamar blue (Gray and Wickstrom, 1996). Results for each test agent were reported as the percent growth of the treated cells compared to the untreated controls. Compounds which reduce the growth of any one of the cell lines to approximately 32 % or less (negative numbers indicate cell death) were passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. All compounds were tested at a final concentration of 10^{-4} M.

General synthesis

Synthesis of 3′,4′-diaryl-4′H-spiro[isothiochromene-3,5′-isoxazol]-4(1H)-ones: ***10–27***

In an erlenmeyer equipped with a bulb for addition, a mixture of 10 mmol of the 3-arylideneisothiochroman-4-one **1–5** and 12 mmol of oxime **6–9** in 20 mL of chloroform was placed in an ice-salt bath under magnetic stirring. Then, 10 mL of a sodium hypochlorite solution (NaOCl, 18°) was added and stirring was maintained for one more hour. The organic phase was separated, washed with water, and dried on anhydrous sodium sulfate. The obtained residue after evaporation of the solvent was recrystallized from ethanol. This series was previously well characterized by ^{1}H, ^{13}C NMR, MS, and elemental analyses (Bennani *et al.*, 2007a; 2004; Al Houari *et al.*, 2008).

Synthesis of 3,4-di-substituted-isothiochromeno[3,4-e][1,2]oxazines: ***28–45***

A volume of 10 mL of concentrated hydrochloric acid was carefully added, drop by drop, to a solution of 2 mmol of compounds **10–27** in 15 mL of EtOH. Agitation at refluxing solvent was maintained for 10 h after the addition. The organic phase was concentrated to half initial volume. White solide is obtained and recuperated by filtration, washed several times with water, and was recrystallized from ethanol.

3,4-diphenylisothiochromeno[3,4-e][1,2]oxazine: ***28*** White powder. Yield = 52 %. M.p. = 156–157 °C. IR (KBr, υ cm^{-1}): 3140 (=CH), 2990 (CH), 1540 (C=C), 1480 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 2$), 7.70 (s, 1H, H^6), 7.10–7.60 (m, 13H, Haromat). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: C^{10b} (163.00), C^3 (161.50), C^6 (120.80), C–H aromat. (120.10; 122.20; 124.30; 125.95; 128.30; 128.50; 128.90; 129.30; 129.50; 131.00), C aromat. (114.80; 118.40; 128.70; 130.10; 136.70; 138.60). MS (EI, 70 eV): $[M]^{+\cdot} = 353$ [$C_{23}H_{15}NOS$, (100 %)]. Elemental analysis for $C_{23}H_{15}NOS$: Cal. (found): C 78.16 (77.98); H 4.28 (4.23); N 3.96 (4.17).

3-(4-methylphenyl)-4-phenylisothiochromeno[3,4-e][1,2]oxazine: ***29*** White powder. Yield = 55 %. M.p. = 163–165 °C. IR (KBr, υ cm^{-1}): 3139 (=CH), 2991 (CH), 1541 (C=C), 1479 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.10–7.70 (m, 12H, Haromat), 2.40 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: Methyl (21.40), C^6 (120.80), C^3 (161.50), C^{10b} (162.90), C–H aromat. (122.15; 122.30; 124.40; 126.00; 128.30; 128.70; 129.30; 129.35; 131,00), C aromat. (114.80; 118.60; 126.00; 130.30; 136.70; 138.60; 139.60). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 367 [$C_{24}H_{17}NOS$, (19 %)], 234.20 (100 %). Elemental analysis for $C_{24}H_{17}NOS$: Cal. (found): C 78.45 (78.37); H 4.66 (4.58); N 3.81 (3.78).

3-(4-methoxyphenyl)-4-phenylisothiochromeno[3,4-e][1,2]oxazine: ***30*** White powder. Yield = 61 %. M.p. = 148–150 °C. IR (KBr, υ cm^{-1}): 3128 (=CH), 2985 (CH), 1542 (C=C), 1483 (–C=N), ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.25 (dd, 1H, H^9, $J_o = 8.5$; $J_m = 2$), 7.65 (s, 1H, H^6), 6.80–7.06 (m, 13H, Haromat), 3.70 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: Methoxy (55.30), C^6 (120.80), C^3 (161.20), C^{10b} (162.90), C–H aromat. (114.10; 122.20; 122.40; 124.40; 126.00; 128.75; 129.40; 129.80; 131.20), C aromat. (114.80; 130.50; 131.20; 136.75; 138.70; 160.65). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 383 [$C_{24}H_{17}NO_2S$, (71 %)]; 234.15 (100 %). Elemental analysis for $C_{24}H_{17}NOS$: Cal. (found): C 75.17 (74.93); H 4.47 (4.42); N 3.65 (3.57).

3-(4-nitrophenyl)-4-phenylisothiochromeno[3,4-e][1,2]oxazine: ***31*** White powder. Yield = 50 %. M.p. = 202–204 °C. IR (KBr, υ cm^{-1}): 3139 (=CH), 2991 (CH), 1543 (C=C), 1484 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 8.0$; $J_m = 1$), 7.75 (s, 1H, H^6), 7.10–8.16 (m, 12H, Haromat). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: C^6 (121.65), C^3 (159.70), C^{10b} (164.00), C–H aromat. (122.10; 122.30; 123.80; 124.60; 126.40; 129.20; 129.40; 129.75; 131.00), C aromat. (114.60; 117.85; 129.30; 135.30; 137.00; 138.70; 148.40). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 398 [$C_{23}H_{14}N_2O_3S$, (74 %)]; 89.15 (100 %). Elemental analysis for $C_{23}H_{14}N_2O_3S$: Cal. (found): C 69.33 (69.27); H 3.54 (3.47); N 7.03 (6.96).

3-(4-chlorophenyl)-4-phenylisothiochromeno[3,4-e][1,2]oxazine: ***32*** White powder. Yield = 55 %. M.p. = 168–170 °C. IR (KBr, υ cm^{-1}): 3141 (=CH), 2988 (CH), 1537 (C=C), 1481 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.25 (dd, 1H, H^9, $J_o = 8.5$; $J_m = 1$), 7.65 (s, 1H, H^6), 7.10–7.70 (m, 12H, Haromat). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: C^6 (121.10), C^3 (160.60), C^{10b} (163.30), C–H aromat. (121.20; 122.25; 122.30; 124.50; 126.20; 127.50; 127.65; 127.85; 128.70; 128.85), C aromat. (114.60; 118.30; 126.15; 128.75; 135.70; 136.85; 136.70). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 387 [$C_{23}H_{14}ClNOS$, (74 %)], 89.15 (100 %). Elemental analysis for $C_{23}H_{14}ClNOS$: Cal. (found): C 71.22 (70.97); H 3.64 (3.59); N 3.61 (3.57).

4-(4-methylphenyl)-3-phenylisothiochromeno[3,4-e][1,2]oxazine: ***33*** White powder. Yield = 60 %. M.p. = 138–141 °C. IR (KBr, υ cm^{-1}): 3141 (=CH), 2991 (CH), 1542 (C=C), 1481 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm:

8.35 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.15–7.60 (m, 12H, Haromat), 2.50 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: Methyl (21.50), C^6 (120,80), C^3 (161.60), C^{10b} (163.00), C–H aromat. (122.20; 122.50; 124.50; 126.00; 128.40; 128.50; 129.50; 130.10; 130.90), C aromat. (115.00; 118.70; 127.00; 129.05; 136.70; 138.60; 140.80). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 367 [$C_{24}H_{17}NOS$, (31 %)], 236.10 (100 %). Elemental analysis for $C_{24}H_{17}NOS$: Cal. (found): C 78.45 (78.12); H 4.66 (4.53); N 3.81 (3.76).

3,4-bis(4-methylphenyl)isothiochromeno[3,4-e][1,2]oxazine: ***34*** White powder. Yield = 70 %. M.p. = 163–165 °C. IR (KBr, υ cm^{-1}): 3137 (=CH), 2991 (CH), 1542 (C=C), 1481 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.10–7.60 (m, 11H, Haromat), 2.50 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: $2CH_3$ (21.40, 21.50), C^6 (120.60), C^3 (161.50), C^{10b} (162.80), C–H aromat. (122.10; 122.40; 124.30; 125.90; 128.20; 129.25; 130.05; 130.90), C aromat. (114.80; 118.80; 126.10; 127.15; 136.70; 138.55; 138.60; 139.50). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 381 [$C_{25}H_{19}NOS$, (33 %)], 250.10 (100 %). Elemental analysis for $C_{25}H_{19}NOS$: Cal. (found): C 78.71 (78.65); H 5.02 (4.97); N 3.67 (3.63).

3-(4-methoxyphenyl)-4-(4-methylphenyl)isothiochromeno [3,4-e][1,2]oxazine: ***35*** White powder. Yield = 65 %. M.p. = 181–183 °C. IR (KBr, υ cm^{-1}): 31437 (=CH), 2989 (CH), 1538 (C=C), 1481 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 6.80–7.60 (m, 11H, Haromat), 3.80 (s, 3H, O–CH_3), 2.45 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: CH_3 and OCH_3 (21.50, 55.20), C^6 (120.60), C^3 (161.10), C^{10b} (162.80), C–H aromat. (114.00; 122.12; 122.40; 124.30; 125.90; 128.70 130.20; 131.00), C aromat. (114.70; 118.80; 121.40; 127.25; 136.70; 138.60 160.60). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 397 [$C_{25}H_{19}NO_2S$, (74 %)], 248.25 (100 %). Elemental analysis for $C_{25}H_{19}NO_2S$: Cal. (found): C 75.54 (75.46); H 4.82 (4.78); N 3.52 (3.48).

4-(4-methylphenyl)-3-(4-nitrophenyl)isothiochromeno[3,4-e] [1,2]oxazine: ***36*** White powder. Yield = 56 %. M.p. = 219–223 °C. IR (KBr, υ cm^{-1}): 3141 (=CH), 2991 (CH), 1539 (C=C), 1481 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.75 (s, 1H, H^6), 7.15–8.20 (m, 11H, Haromat), 2.50 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: CH_3 (21.50), C^6 (121.45), C^3 (159.70), C^{10b} (163.90), C–H aromat. (122.15; 122.20; 123.70; 124.50; 126.30; 129.10; 130.50; 130.70), C aromat. (114.55; 117.95; 126.15;135.40; 136.90; 138.60; 139.30; 148.30). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 412 [$C_{24}H_{16}N_2O_3S$, (49 %)], 221 (100 %). Elemental analysis for $C_{24}H_{16}N_2O_3S$: Cal. (found): C 69.89 (69.75); H 3.91 (3.87); 6.79 (6.73).

3-(4-chlorophenyl)-4-(4-methylphenyl)isothiochromeno [3,4-e][1,2]oxazine: ***37*** White powder. Yield = 50 %. M.p. = 133–135 °C. IR (KBr, υ cm^{-1}): 3141 (=CH), 2992 (CH), 1541 (C=C), 1479 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.10–7.60 (m, 11H, Haromat), 2.50 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: CH_3 (21.50), C^6 (120.95), C^3 (160.60), C^{10b} (163.25), C–H aromat. (122,20; 122,30; 124,40; 126,10; 128,80; 129,60; 130,25; 130,80), C aromat. (114.60; 118.45; 126,70; 27,50; 135.60; 136.75; 138.60; 138.85). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 401 [$C_{24}H_{16}ClNOS$, (3 %)], 221 (100 %). Elemental analysis for $C_{24}H_{16}ClNOS$: Cal. (found): C 71.72 (71.68); H 4.01 (3.97); N 3.49 (3.45).

4-(4-methoxyphenyl)-3-phenylisothiochromeno[3,4-e][1,2] oxazine: ***38*** White powder. Yield = 60 %. M.p. = 138–140 °C. IR (KBr, υ cm^{-1}): 3139 (=CH), 2991 (CH), 1541 (C=C), 1482 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.35 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.00–7.70 (m, 13H, Haromat), 3.90 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: OCH_3 (55.30), C^6 (120.70), C^3 (161.50), C^{10b} (163.10), C–H aromat. (114.80; 120.70; 122.10; 122.40; 124.35; 125.95; 128.32; 128.50; 129.45), C aromat. (114.45; 118.70; 122.05; 129.00; 136.65; 138.55; 159.95). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 383 [$C_{24}H_{17}NO_2S$, (7 %)]; 222 (100 %). Elemental analysis for $C_{24}H_{17}NO_2S$: Cal. (found): C 75.17 (74.95); H 4.47 (4.42); N 3.65 (3.61).

4-(4-methoxyphenyl)-3-(4-methylphenyl)isothiochromeno [3,4-e][1,2]oxazine: ***39*** White powder. Yield = 65 %. M.p. = 182–184 °C. IR (KBr, υ cm^{-1}): 3138 (=CH), 2991 (CH), 1541 (C=C), 1482 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.65 (s, 1H, H^6), 6.85–7.55 (m, 11H, Haromat), 3.80 (s, 3H, O–CH_3), 2.45 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: (OCH_3 (55.25), CH_3 (21.50), C^6 (120.60), C^3 (161.15), C^{10b} (162.80), C–H aromat. (114.00; 122.15; 122.40; 124.35; 125.90; 129.90; 130.15; 131.00), C aromat. (114.70; 118.80; 121.45; 127.25; 138.10; 138.60; 160.55). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 397 [$C_{25}H_{19}NO_2S$, (39 %)], 248 (100 %). Elemental analysis for $C_{25}H_{19}NO_2S$: Cal. (found): C 75.54 (75.46); H 4.82 (4.78); N 3.52 (3.47).

3,4-bis(4-methoxyphenyl)isothiochromeno[3,4-e][1,2]oxazine: ***40*** White powder. Yield = 60 %. M.p. = 133–135 °C. IR (KBr, υ cm^{-1}): 3139 (=CH), 2989 (CH), 1541 (C=C), 1482 (–C=N). ^1H NMR (250 MHz, $CDCl_3$) δ ppm:

8.35 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 6.60–7.55 (m, 11H, Haromat), 3.90 (s, 3H, O–CH_3), 3.80 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: 2OCH_3 (55.25, 55.30), C^6 (120.55), C^3 (161.10), C^{10b} (162.90), C–H aromat. (113.95; 114.85; 122.00; 122.40; 124.35; 125.90; 129.65; 132.30), C aromat. (114.25; 118.90; 121.40; 122.25; 136.60; 138.55; 159.90; 160.50). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 413 [$C_{25}H_{19}NO_3S$, (28 %)]; 266.20 (100 %).

Elemental analysis for $C_{25}H_{19}NO_3S$: Cal. (found): C 72.62 (72.55); H 4.63 (4.57); N 3.39 (3.34).

4-(4-methoxyphenyl)-3-(4-nitrophenyl)isothiochromeno [3,4-e][1,2]oxazine: ***41*** White powder. Yield = 50 %. M.p. = 191–193 °C. IR (KBr, υ cm^{-1}): 3140 (=CH), 2991 (CH), 1540 (C=C), 1481 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.75 (s, 1H, H^6), 7.00–8.20 (m, 11H, Haromat), 3.90 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: OCH_3 (55.35), C^6 (121.45), C^3 (160.30), C^{10b} (164.10), C–H aromat. (115.20; 122.15; 122.23; 123.75; 124.50; 126.35; 129.10; 132.15), C aromat. (114.20; 115.20 118.05; 121.50; 135.40; 136.80; 138.60; 148.25; 159.70). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 428 [$C_{24}H_{16}N_2O_4S$, (13 %)], 221 (100 %). Elemental analysis for $C_{24}H_{16}N_2O_4S$: Cal. (found): C 67.28 (67.17); H 3.76 (3.65); N 6.54 (6.49).

3-(4-chlorophenyl)-4-(4-methoxyphenyl)isothiochromeno [3,4-e][1,2]oxazine: ***42*** White powder. Yield = 57 %. M.p. = 162–164 °C. IR (KBr, υ cm^{-1}): 3141 (=CH), 2990 (CH), 1542 (C=C), 1480 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.30 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.70 (s, 1H, H^6), 7.00–7.60 (m, 11H, Haromat), 3.90 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: OCH_3 (55.55), C^6 (121.81), C^3 (161.10), C^{10b} (163.12), C–H aromat. (116.13; 121.93; 123.03; 123.95; 124.55;5; 148.28; 159.79). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 417 [$C_{24}H_{16}ClNO_2S$, (39 %)], 248.15 (100 %). Elemental analysis for $C_{24}H_{16}ClNO_2S$: Cal. (found): C 68.98 (68.87); H 3.86 (4.78); N 3.35 (3.32).

3-(4-methylphenyl)-4-(4-nitrophenyl)isothiochromeno[3,4-e][1,2]oxazine: ***43*** White powder. Yield = 50 %. M.p. = 188–190 °C. IR (KBr, υ cm^{-1}): 3142 (=CH), 2991 (CH), 1540 (C=C), 1480 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.25 (dd, 1H, H^9, $J_o = 9$; $J_m = 2$), 7.80 (s, 1H, H^6), 7.00–8.30 (m, 11H, Haromat), 2.40 (s, 3H, CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: CH_3 (21.40), C^6 (121.60), C^3 (161.55), C^{10b} (163.10), C–H aromat. (121.90; 122.30; 124.35; 124.60; 126.50; 128.35; 129.50; 131.90), C aromat. (113.00; 117.20; 125.20; 137.10; 137.50; 138.85; 140.10). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 412 [$C_{24}H_{16}N_2O_3S$] (100 %). Elemental analysis for $C_{24}H_{16}N_2O_3S$: Cal. (found): C 69.89 (69.81); H 3.91 (3.89); N 6.79 (6.75).

3-(4-methoxyphenyl)-4-(4-nitrophenyl)isothiochromeno [3,4-e][1,2]oxazine: ***44*** White powder. Yield = 60 %. M.p. = 196–198 °C. IR (KBr, υ cm^{-1}): 3140 (=CH), 2991 (CH), 1540 (C=C), 1481 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.50 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.80 (s, 1H, H^6), 6.90–8.30 (m, 11H, Haromat), 3.85 (s, 3H, O–CH_3). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: OCH_3 (55.35), C^6 (121.60), C^3 (161.20), C^{10b} (163.10), C–H aromat. (122.30; 122.40; 124.50; 124.70; 126.70; 129.20; 129.70; 131.90), C aromat. (112.85; 117.20; 120.30; 137.00; 137.60; 138.80; 147.75; 160.85). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 428 [$C_{24}H_{16}N_2O_4S$ (8 %)]. Elemental analysis for $C_{24}H_{16}N_2O_4S$: Cal. (found): C 67.28 (67.17); H 3.76 (3.69); N 6.54 (6.51).

3-(4-chlorophenyl)-4-(4-nitrophenyl)isothiochromeno[3,4-e] [1,2]oxazine: ***45*** White powder. Yield = 50 %. M.p. = 176–178 °C. IR (KBr, υ cm^{-1}): 3139 (=CH), 2990 (CH), 1540 (C=C), 1478 (–C=N). ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm: 8.50 (dd, 1H, H^9, $J_o = 9$; $J_m = 1$), 7.80 (s, 1H, H^6), 7.15–8.30 (m, 11H, Haromat). ^{13}C NMR (250 MHz, $CDCl_3$) δ ppm: C^6 (121.80), C^3 (160.60), C^{10b} (163.60), C–H aromat. (121.90; 122.40; 124.50; 124.70; 126.70; 129.20; 129.70; 131.90), C aromat. (112.80; 116.85; 124.50; 136.25; 137.00; 137.20; 138.90; 147.90). MS (EI, 70 eV): $[M]^{+\cdot} = m/z$: M = 432 [$C_{23}H_{13}ClN_2O_3S$] (3 %), 118 (100 %). Elemental analysis for $C_{23}H_{13}ClN_2O_3S$: Cal. (found): C 63.82 (63.76); H 3.03 (3.01); N 6.47 (6.45).

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