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Synthesis of acridinyl-thiazolino derivatives and their evaluation for anti-inflammatory, analgesic and kinase inhibition activities

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Abstract—Variety of *N*-(4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-ylidene)-2,4(un)substituted acridin-9-amine (**4a**–**o**) and 1-[(2,4-(un)substituted acridin-9-yl)-3-(4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-ylidene)]isothiourea (**5a**–**h**) derivatives have been synthesized by condensation of 4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-imine (**3a**–**g**) with 9-chloro-2,4-(un)substituted acridine (**1a**–**c**) and 9-isothiocyanato-2,4-(un)substituted acridine (**2a**–**d**), respectively. All these compounds were characterized by correct ^{1}H NMR, FT-IR, MS and elemental analyses. These compounds were screened for anti-inflammatory, analgesic and kinase (CDK1, CDK5 and GSK3) inhibition activities. Some compounds exhibited good anti-inflammatory (25–32%) and potent analgesic (50–75%) activities, at 50 mg/kg p.o. A compound, **4o** (R_1 = H, R_2 = OCH_3, R_3 = CH_3, R_4 = CH_3, R_5 = H) exhibited moderate CDK1 (IC_{50} = 8.5 μM) inhibition activity.

1. Introduction

Acridine and its derivatives, well known as DNA intercalates, have been widely studied from a variety of viewpoints, such as synthesis,[1] physicochemical properties,[2] structural requirements[3] and biological activities.[4] Due to a polycyclic planar structure, acridine moiety interacts with DNA by intercalation between base pairs and interferes with essential metabolic processes.[5] On the basis of the DNA-binding properties of peptide–acridine hybrids, Martelli et al.[6] offered DNA-binding models in which the acridine moiety protrudes slightly outside the double helix but remains more or less parallel to the plane of the base pairs.

Apart from anti-cancer activity,[7] variety of natural and synthetic acridine derivatives have also been tested for anti-malarial,[8] anti-inflammatory[9] and analgesic[10] activities and some of them have been approved for chemotherapy. The mechanisms of action of acridine moiety in the chemotherapy revealed, interference of acridine with the activity of topoisomerases and telomerases enzymes[5] and other cellular targets such as cyclin-dependent kinase.[11] An automated synthesis of 5′-acridine linked oligothymidylates using phosphoramidite linked acridine compounds are reported in the literature.[12] Amino-acridine conjugates play an important role as biochemical probes.[13] An effect of acridine derivatives on DNA synthesis raised the hypothesis that acridine moieties secondary effect on biochemical pathways, including protein and lipid metabolism exists, which suggest that acridine derivatives could be considered as multitarget drugs.[14]

The above shortcomings have motivated our search and also in continuation with our search[15] for biologically active novel compounds to be used in place of, the existing anti-inflammatory drugs having major side effects such as peptic-ulcer formation and gastro-intestinal damage.[16] It was considered worthwhile to synthesize variety of acridinyl-thiazolino derivatives and to study their anti-inflammatory, analgesic and kinase (CDK1, CDK5 and GSK3) inhibition activities.

Keywords: Synthesis; Acridinyl-thiazolino derivatives; Anti-inflammatory; Analgesic; CDK1 inhibitor.
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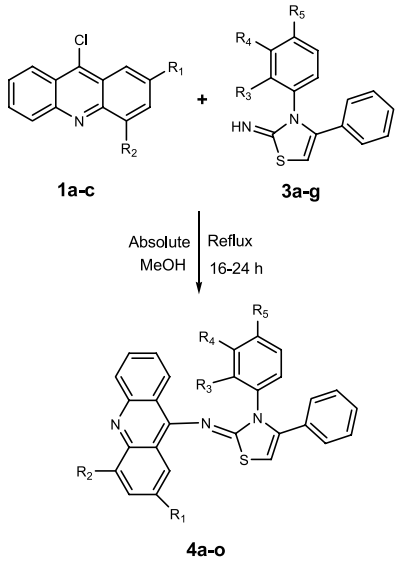
2. Results and discussion

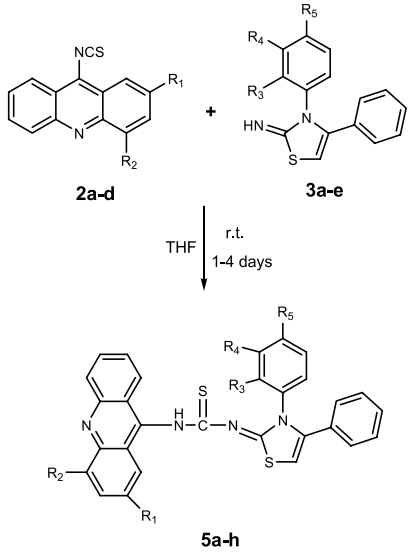
2.1. Chemistry

N-[4-Phenyl-3-(2′,3′,4′(un)substituted phenyl) thiazol-2(3*H*)-ylidene]-2,4(un)substituted acridin-9-amine (**4a**–**o**) compounds were synthesized by the reaction of 4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-imines (**3a**–**g**) and 9-chloro-2,4(un)substituted acridine (**1a**–**c**) in refluxing absolute methanol for 16–24 h (Scheme 1). The condensed compounds (**4a**–**o**) were purified by silica gel column chromatography or by recrystallization with appropriate solvent. The structures assigned for all the synthesized compounds (**4a**–**o**) were fully supported by their correct FT-IR, ^{1}H NMR, MS and elemental analyses (CHNS) and their details are presented in experimental section. The requisite, 9-chloro-2,4-(un)substituted acridine (**1a**–**c**) compounds were synthesized by condensation of *N*-aryl anthranalic acid[17] with phosphorousoxychloride by a reported method.[18] The second precursor, 4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-imine (**3a**–**g**) were prepared by the condensation of phenacyl-thiocyanate with variety of amine hydrochloride as reported by Mahajan et al.[19]

A different series of compounds namely, 1-[(2,4-(un)substituted acridin-9-yl)-3-(4-phenyl-3-2′,3′,4′(un)-substituted phenyl)thiazol-2(3*H*)-ylidene]isothiourea (**5a**–**h**) were synthesized by condensation of 4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-imine (**3a**–**e**) with 9-isothiocyanto 2,4(un)substituted acridine (**2a**–**d**) in tetrahydrofuran (THF) at room temperature (rt) for one to four days (Scheme 2). The precursors 9-isothiocyanato-2,4(un)substituted acridine (**2a**–**d**) were synthesized by a procedure reported in the literature.[20] All the synthesized compounds were purified either by silica gel column chromatography or by recrystallization. The structures assigned to compounds **5a**–**h** were fully supported by correct spectroscopic data reported in the Experimental section (Table 1).



Scheme 1.



Scheme 2.

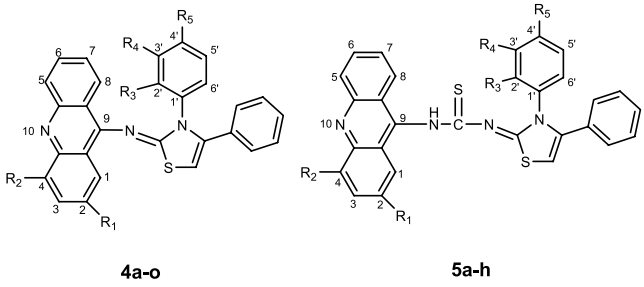


Table 1. Various acridinyl-thiazolino derivatives with different substituents

Compound	R_1	R_2	R_3	R_4	R_5
4a	OCH_3	H	H	H	OCH_3
4b	OCH_3	H	H	H	Cl
4c	OCH_3	H	H	H	CH_3
4d	OCH_3	H	CH_3	CH_3	H
4e	H	CH_3	OCH_3	H	H
4f	H	CH_3	H	H	OCH_3
4g	H	CH_3	H	H	Cl
4h	H	CH_3	H	H	CH_3
4i	H	CH_3	CH_3	CH_3	H
4j	H	OCH_3	OCH_3	H	H
4k	H	OCH_3	NO_2	H	H
4l	H	OCH_3	H	NO_2	H
4m	H	OCH_3	H	H	OCH_3
4n	H	OCH_3	H	H	Cl
4o	H	OCH_3	CH_3	CH_3	H
5a	H	H	H	H	H
5b	H	H	CH_3	H	H
5c	CH_3	H	H	H	H
5d	CH_3	H	H	H	CH_3
5e	H	CH_3	H	H	H
5f	H	CH_3	H	H	CH_3
5g	H	CH_3	H	H	OCH_3
5h	H	OCH_3	H	H	H

FT-IR spectra of compounds **4a–o** exhibited a strong absorption bands in the region 1609–1630 cm^{-1} corresponding to >C=N– group stretching vibrations and absorption in the region 1580–1402 cm^{-1} attributed to aromatic group. The absorption bands for compounds **5a–h** at 3459–3300, 1635–1594 and 1218–1208 cm^{-1} corresponded to N–H, >C=N– and >C=S stretching vibration, respectively. Peaks in the region 1574–1464 cm^{-1} were attributed for aromatic group. The ^1H NMR spectra for compounds **4a–o** showed, a characteristic singlet for a proton of thiazoline ring in the region δ 6.09–6.68 ppm. The protons of aromatic ring attached to N-atom of the thiazoline ring appeared in the region δ 6.35–6.94. Whereas the protons of aromatic ring (attached to C-atom of the thiazoline ring) were up field δ 7.00–7.30 as compared to the proton at C-5 and C-4 atom of acridine ring appeared in the region δ 7.9–8.23 because of the presence of ring nitrogen atom. The signals of protons attached to C-1, C-2, C-3, C-6, C-7 and C-8 of acridine ring appeared at δ 7.0–7.9. Similar signals were defined for the protons of compounds **5a–h** in the ^1H NMR, only the significant difference was appearance of a broad peak (HN–) in the region δ 8.16–11.32. MS analysis of the compounds **4a–o** showed M^+ or MH^+ ion peaks and appropriate fragmentation pattern (in Experimental section). However EI-MS or HR-MS of compounds **5a–h** did not show M^+ ion peak indicating fragile nature of these compounds, nonetheless the fragmented peaks found in MS were in agreement with the theoretical metastable ions. Our earlier report[21] also supports the fragile nature of 1-[(2,4-(un)substituted acridin-9-yl)-3-(4-phenyl-3-2′,3′,4′(un)- substituted phenyl)thiazol-2(3*H*)-ylidene]isothiourea derivatives. Elemental analyses of the compounds **4a–o** and **5a–h** were found in agreement with the theoretically calculated values.

2.2. Biological activity

The acridinyl-thiazolino derivatives **4a–o** and **5a–h** have been tested for (i) anti-inflammatory activity in the carrageenin- induced paw oedema model at 50 mg/kg p.o., (ii) analgesic activity in the phenylquinone writhing assay at 50 mg/kg p.o. and (iii) cyclin-dependent kinase (CDK1 and CDK5) and glycogen synthase kinase (GSK3) inhibitory activity. Results of these biological activities are summarized in Table 2.

Table 2 suggests that compounds **4j**, **4k** and **5h**, each one with $-OCH_3$ group at R_2 position of acridine ring and $-OCH_3$, $-NO_2$ and H groups, respectively at R_3 position in the phenyl ring are favourable for anti-inflammatory activity of acridinyl-thiazolino derivatives. The potent analgesic activity of compounds **4o** and **5d** is neither correlate with the different substituents at R_2 position of acridine ring nor with that of R_3 and R_4 positions of the phenyl ring. So at this stage no conclusion can be drawn about the analgesic nature of these compounds.

Although compounds **4a–n** and **5a–h** have not shown any activity for kinase inhibition, the compound **4o** with $R_1 = H$, $R_2 = OCH_3$, $R_3 = CH_3$, $R_4 = CH_3$, $R_5 = H$ exhibited moderate activity against CDK1 (IC_{50} = 8.5 μM). A structural comparison of this active compound (**4o**) with its related analogues (**4j–n**) indicates that the environment around the phenyl ring favours the ability of this compound to evoke the bioresponse. Our primary observations also indicate importance of both R_3 and R_4 positions with $-CH_3$ group to interact with some active sites of CDK1. However the significance of $-OCH_3$ group at R_2 position of acridine ring cannot be diminished. The literature[22] also supports

Table 2. Anti-inflammatory, analgesic and kinase (CDK1, CDK5 and GSK3) inhibition activities of compounds **4a–o** and **5a–h**

Compound	Anti-inflammatory (%)	Analgesic (%)	Kinase IC_{50} (μM)		
	50 mg/kg p.o.	50 mg/kg p.o.	CDK1	CDK5	GSK3
4a	19.2	—	—	>10	>10
4b	28.6	25	—	>10	>10
4c	15.3	—	>10	—	>10
4d	—	—	>10	—	>10
4e	25.6	42	—	>10	>10
4f	26.4	28	—	>10	>10
4g	18.5	—	—	>10	>10
4h	26.2	32	>10	—	>10
4i	27.4	35	>10	—	>10
4j	**29.1**	25	>10	—	>10
4k	**31.2**	26	>10	—	>10
4l	23.2	40	>10	—	>10
4m	28.4	30	>10	—	>10
4n	—	—	>10	—	>10
4o	0.0	**75**	**8.5**	—	>10
5a	20.4	—	>10	—	>10
5b	0.0	25	—	>10	>10
5c	12.5	—	>10	—	>10
5d	0.0	**50**	—	>10	>10
5e	—	—	—	>10	>10
5f	14.6	—	>10	>10	>10
5g	—	—	—	>10	>10
5h	**32.5**	—	>10	>10	>10
Ibuprofen	38.4	50	—	—	—

better biological activity of acridine derivatives with $-OCH_3$ group rather than $-CH_3$ group at R_2 position.

3. Conclusion

In summary, we have synthesized twenty-three novel acridinyl-thiazolino derivatives by a simple but efficient single-pot reaction. The compounds **4j**, **4k** and **5h** have shown good anti-inflammatory activity ($\geqslant 30\%$) whereas compounds **4o** and **5d** showed potent analgesic activity ($\geqslant 50\%$). Although, other acridinyl-thiazolino analogues have shown moderate to nil anti-inflammatory activities, they are helpful to facilitate the structure–activity relationship. A compound **4o** with moderate activity against CDK1 ($IC_{50} = 8.5\ \mu M$) suggest that there is some scope to investigate novel analogues of this important class of compounds as prospective CDK1 inhibitors.

4. Experimental

4.1. General

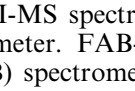
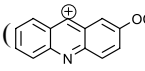
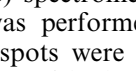
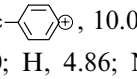
Melting points (mp) were uncorrected and obtained on a capillary JSGW apparatus. IR spectra were recorded using a Perkin Elmer 1600FT Spectrophotometer and only characteristic peaks are reported. ^1H NMR spectra were measured on a Bruker WH-300 Spectrometer in a ca. 5–15% (w/v) solution in DMSO-d_6. Chemical shifts (δ) are reported in parts per million (ppm) of the applied field by using TMS as internal standard. The HR-MS peak measurements were made by comparison with perfluorotributylamine using an AEI MS-9 double focusing high resolution mass spectrometer at a resolving power 15000. The EI-MS spectra were recorded on VG-70-S mass spectrometer. FAB-MS were reordered on Jeol SX–120 (FAB) spectrometer. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapours or by irradiation with ultraviolet light (254 nm). Column chromatography was performed by using Qualigen silica gel (60–120 mesh).

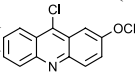
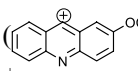
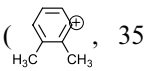
4.2. Synthesis of *N*-(4-phenyl-3-(2′,3′,4′-(un)substituted phenyl)thiazol-2(3*H*)-ylidene)-2,4(un)substituted acridin-9-amine: a general method for preparation of 4a–o

2,4(Un)substituted-9-chloroacridine (1 mmol) (**1a**–**c**) was dissolved in absolute methanol (~50 mL) and to it was added 4-phenyl-3-(2′,3′,4′-(un)substituted phenyl) thiazol-2-(3*H*)imine (1 mmol) (**3a**–**g**). The reaction contents were heated under reflux for 16–24 h and after completion of reaction solvent was removed under reduced pressure. The solid residue was suspended in 10 mL aqueous sodium carbonate solution (10%) and stirred for 20 min. It was then filtered, washed with water and air dried to give crude products. The crude product was purified either by column chromatography or recrystallization, to give pure condensed product (**4a**–**o**).

4.2.1. *N*-(4-Phenyl-3-(4′-methoxyphenyl)thiazol-2(3*H*)-ylidene)-2-methoxy acridin-9-amine (4a). Solvent of elution, $CHCl_3$/EtOAc (4:1); Orange solid (0.211 g, 35%); mp 188–190 °C; IR (KBr) ν_{max}: 1611, 1511, 1466, 1258, 1045 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.51 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 6.24 (s, 1H, >C=CH–), 6.75–6.81 (dd, 2H, Ar), 6.94 (s, 1H, Ar), 7.07 (s, 5H, Ar), 7.24–7.40 (m, 4H, Ar), 7.63 (m, 1H, Ar), 7.76–7.82 (t, 3H, Ar); FAB-MS 490.1101 (MH^+, 100%). Anal. Calcd for $C_{30}H_{23}N_3SO_2$: C, 73.61; H, 4.70; N, 8.58; S, 6.54. Found: C, 73.34; H, 4.86; N, 8.28; S, 6.49.

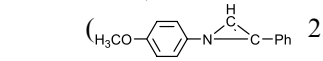
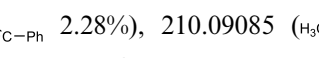
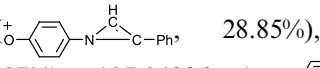
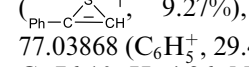
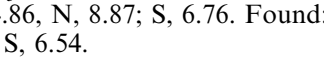
4.2.2. *N*-(4-Phenyl-3-(4′-chloro phenyl)thiazol-2(3*H*)-ylidene)-2-methoxy acridin-9-amine (4b). Solvent of elution, $CHCl_3$/EtOAc (1:1); Yellow solid (0.121 g, 30%); mp 63–65 °C; IR (KBr) ν_{max}: 1620, 1553, 1482, 1288, 1085 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.70 (s, 3H, OCH_3), 6.40 (s, 1H, >C=CH–), 6.70–6.90 (m, 3H, Ar), 7.10–7.40 (m, 10H, Ar), 7.60 (t, 1H, Ar), 7.80 (t, 1H, Ar); 7.95 (d, 1H, Ar); HR-MS: found 495.09855 (M^+, 7.66) Calcd for $C_{29}H_{20}N_3OSCl^{37}$ 495.09860, 493.10015 (M^+, 18.47) Calcd for $C_{29}H_{20}N_3OSCl^{35}$ 493.10156, 480.07421 (M^+-CH_3, 2.03%), 478 (M^+-CH_3, 4.88%). Anal. Calcd for $C_{29}H_{20}N_3SOCl$: C, 70.58; H, 4.05; N, 8.51; S, 6.49. Found: C, 70.24; H, 4.18; N, 8.34; S, 6.58.

4.2.3. *N*-(4-Phenyl-3-(4′-methyl phenyl)thiazol-2(3*H*)-ylidene)-2-methoxy acridin-9-amine (4c). Solvent of elution, $CHCl_3$/EtOAc (4:1); Yellow solid (0.262 g, 45%); mp 173–175 °C; IR (KBr) ν_{max}: 1612, 1555, 1507, 1248, 1089 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.29 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.48 (s, 1H, >C=CH–), 7.15 (d, 1H, Ar), 7.22–7.28 (d + s, 6H, Ar), 7.45–7.54 (m, 5H, Ar), 7.68–7.774 (m, 1H, Ar), 7.98–8.05 (q, 3H, Ar); EI-MS: 473 (M^+, 100%), 458 (M^+-CH_3, 14.6%), 265 ( , 1.0%), 208 ( , 0.7%), 194 ( , 18.2%), 91 ( , 10.0%). Anal. Calcd for $C_{30}H_{23}N_3SO$: C, 76.10; H, 4.86; N, 8.87;S, 6.76. Found: C, 76.53; H, 4.53; N, 8.58; S, 6.45.

4.2.4. *N*-(4-Phenyl-3-(2′,3′-dimethyl phenyl)thiazol-2(3*H*)-ylidene)-2-methoxy acridin-9-amine (4d). Solvent of elution, CCl_4/EtOAc (9:1); Red solid (0.300 g, 50%); mp 95–97 °C; IR (KBr) ν_{max}: 2945, 1612, 1555, 1259, 1047 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.22–2.26 (2s, 6H, $CH_3 + CH_3$), 3.84 (s, 3H, OCH_3), 6.52 (s, 1H, >C=CH–), 7.05–7.26 (m, 9H, Ar), 7.34–7.53 (m, 3H, Ar), 7.71–7.74 (t, 1H, Ar), 7.95–8.05 (m, 2H, Ar); EI-MS 487 (M^+, 10.7%), 266 ( , 10.7%), 208 ( , 4.7%), 105 ( , 35.9%), 77 ($C_6H_5^+$, 34.2%). Anal. Calcd for $C_{31}H_{25}N_3SO$: C, 76.38; H, 5.13; N, 8.62; S, 6.57. Found: C, 76.08; H, 5.38; N, 8.48; S, 6.61.

4.2.5. *N*-(4-Phenyl-3-(2′-methoxy phenyl)thiazol-2(3*H*)-ylidene)-4-methyl acridin-9-amine (4e). Solvent of crystallization, MeOH; Yellow solid (0.270 g, 65%); mp 235–237 °C; IR (KBr) ν_{max}: 1613, 1545, 1450, 1244,

1087 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.85 (s, 3H, $-CH_3$), 3.91 (s, 3H, OCH_3), 7.14–7.29 (m, 6H, Ar), 7.36–7.41 (t, 2H, Ar), 7.57–7.65 (dd, 2H, Ar), 7.70–7.78 (m, 2H, Ar), 7.90–7.93 (d, 2H, Ar), 8.03–8.13 (m, 2H, Ar), 8.20–8.23 (d, 1H, Ar); FAB-MS 474.1119 (MH^+, 100%). Anal. Calcd for $C_{30}H_{23}N_3SO$: C, 76.10; H, 4.86, N, 8.87; S, 6.76. Found: C, 76.58; H, 4.75; N, 8.98; S, 6.62.

4.2.6. *N*-(4-Phenyl-3-(4′-methoxy phenyl)thiazol-2(3*H*)-ylidene)- 4-methyl acridin-9-amine (4f). Solvent of elution, $CHCl_3$/EtOAc (4:1); Brown solid (0.250 g, 40%); mp 90–92 °C; IR (KBr) ν_{max}: 1609, 1510, 1466, 1258, 1046 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.80 (s, 3H, CH_3), 4.75 (s, 3H, OCH_3), 6.50 (s, 1H, >C=CH–), 7.00 (d, 2H, Ar), 7.10–7.40 (m, 7H, Ar), 7.45–7.54 (m, 2H, Ar), 7.60–7.68 (d, 1H, Ar), 7.74–7.82 (q, 1H, Ar), 7.86–7.92 (d, 1H, Ar), 7.98–8.04 (d, 1H, Ar), 8.08–8.14 (d, 1H,Ar); HR-MS: found 473.15504 (M^+, 48.52%); Calcd for $C_{30}H_{23}N_3SO$ 473.15619, 472.14796 (M^+-H, 21.71), 443.14428 (M^+-HCHO, 3.0%), 223.09889 ( , 2.28%), 210.09085 ( , 60.48%), 209.08403 (*m/z* 210.09085 $-H^+$, 100.00%), 208.07633 ( , 28.85%), 134.01906 ( , 9.27%), 107.04986 ( , 2.19%), 77.03868 ($C_6H_5^+$, 29.41%). Anal. Calcd for $C_{30}H_{23}N_3SO$: C, 76.10; H, 4.86, N, 8.87; S, 6.76. Found: C, 76.25; H, 4.44; N, 8.48; S, 6.54.

4.2.7. *N*-(4-Phenyl-3-(4′-chloro phenyl)thiazol-2(3*H*)-ylidene)-4-methyl acridin-9-amine (4g). Solvent of elution, $CHCl_3$/EtOAc (4:1); Yellow solid (0.147 g, 35%); mp 230–232 °C; IR (KBr) ν_{max}: 1620, 1580, 1469, 1288, 1085 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.41 (s, 3H, CH_3), 6.95–7.11 (m, 7H, Ar), 7.38–7.54 (m, 4H, Ar), 7.72–7.76 (t, 2H, Ar), 7.91–8.05 (m, 4H, Ar); FAB-MS: 478.0602 (MH^+, 100%). Anal. Calcd for $C_{29}H_{20}N_3SCl$: C, 72.87; H, 4.18; N, 8.79; S, 6.70. Found: C, 72.61; H, 4.07; N, 8.78; S, 6.45.

4.2.8. *N*-(4-Phenyl-3-(4′-methyl phenyl)thiazol-2(3*H*)-ylidene)-4-methyl acridin-9-amine (4h). Solvent of elution, $CHCl_3$/EtOAc (9:1); Yellow solid (0.362 g, 60%); mp 222–225 °C; IR (KBr) ν_{max}: 1623, 1559, 1458, 1282, 1074 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.35 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 6.09 (s, 1H, >C=CH–), 7.20–7.50 (m, 11H, Ar), 7.60 (d, 1H, Ar), 7.70–7.80 (m, 1H, Ar), 7.95 (d, 1H, Ar), 8.05 (d, 1H, Ar), 8.20 (d, 1H, Ar); FAB-MS: 458.1287 (MH^+, 100%). Anal. Calcd for $C_{30}H_{23}N_3S$: C, 78.77; H, 5.03; N, 9.91; S, 7.00. Found: C, 78.98; H, 4.85; N, 9.32; S, 6.82.

4.2.9. *N*-(4-Phenyl-3-(2′,3′-dimethyl phenyl)thiazol-2(3*H*)-ylidene)-4-methyl acridin-9-amine (4i). Solvent of elution, $CHCl_3$/EtOAc (4:1); Red solid (0.210 g, 50%); mp 210–212 °C; IR (KBr) ν_{max}: 1612, 1548, 1462, 1288, 1085 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.28 (s, 6H, $CH_3 + CH_3$); 2.37 (s, 3H, CH_3), 6.91–7.08 (m, 10H, Ar), 7.35–7.39 (d, 1H, Ar), 7.45–7.55 (dd, 2H, Ar), 7.68–7.72 (d, 1H, Ar), 7.88–7.92 (d, 1H, Ar), 7.98–8.02 (d, 1H, Ar); FAB-MS: 472.1406 (MH^+, 100%). Anal. Calcd for $C_{31}H_{25}N_3S$: C, 78.98; H, 5.30; N, 8.91; S, 6.79. Found: C, 78.52; H, 5.18; N, 8.80; S, 6.42.

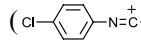
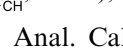
4.2.10. *N*-(4-Phenyl-3-(2′-methoxy phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4j). Solvent of elution, $CHCl_3$/EtOAc (1:1); Brown solid (0.240 g, 40%); mp 198–200 °C; IR (KBr) ν_{max}: 1614, 1544, 1475, 1284, 1044 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.89 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.45(s, 1H, >C=CH–), 7.02–7.79 (m, 14H, Ar), 8.04–8.16 (dd, 2H, Ar); FAB-MS: 490.1147 (MH^+, 100%). Anal. Calcd for $C_{30}H_{23}N_3SO_2$: C, 73.61; H, 4.70; N, 8.58; S, 6.54. Found: C, 73.20; H, 4.49; N, 8.86; S, 6.41.

4.2.11. *N*-(4-Phenyl-3-(2′-nitro phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4k). Solvent of elution, EtOAc: MeOH (9:1); Brown solid (0.180 g, 30%); mp 114–116 °C; IR (KBr) ν_{max}: 1630, 1520, 1425, 1258, 1089 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 4.01 (s, 3H, OCH_3), 6.62 (s, 1H, >C=CH–), 7.06–7.51 (m + s, 8H, Ar), 7.68–8.00 (m, 6H, Ar), 8.13–8.19 (t, 2H, Ar); EI-MS: 504 (MH^+, 100%). Anal. Calcd for $C_{29}H_{20}N_4SO_3$: C, 69.04; H, 3.96; N, 11.11; S, 6.34. Found: C, 69.01; H, 3.98; N, 11.18; S, 6.44.

4.2.12. *N*-(4-Phenyl-3-(3′-nitro phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4l). Solvent of elution, $CHCl_3$; Yellow solid (0.235 g, 40%); mp 152–154 °C; IR (KBr) ν_{max}: 1632, 1520, 1470, 1282, 1024 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 4.00 (s, 3H, OCH_3), 6.52 (s, 1H, >C=CH–), 7.13–7.29 (m + s, 7H, Ar), 7.37–7.43 (t, 1H, Ar), 7.49–7.62 (t + d, 2H, Ar), 7.70–7.81 (m, 2H, Ar), 8.01–8.23 (m, 4H, Ar); EI-MS: 504 (M^+, 75%). Anal. Calcd for $C_{29}H_{20}N_4SO_3$: C, 69.04; H, 3.96; N, 11.11; S, 6.34. Found: C, 69.30; H, 3.73; N, 11.31; S, 6.02.

4.2.13. *N*-(4-Phenyl-3-(4′-methoxy phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4m). Solvent of elution, $CHCl_3$/EtOAc (1:1); Yellow solid (0.240 g, 40%); mp 110–112 °C; IR (KBr) ν_{max}: 1611, 1511, 1258, 1045 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.66 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.47 (s, 1H, >C=CH–), 7.00–7.03 (d, 2H, Ar), 7.14–7.16 (d, 1H, Ar), 7.30 (s, 5H, Ar), 7.36–7.41 (t, 1H, Ar), 7.48–7.60 (m, 4H, Ar), 7.75–7.80 (t, 1H, Ar), 8.03–8.06 (d, 1H, Ar), 8.11–8.14 (d, 1H, Ar); FAB-MS: 490.1075 (MH^+, 100%). Anal. Calcd for $C_{30}H_{23}N_3SO_2$: C, 73.61; H, 4.70; N, 8.58; S, 6.54. Found: C, 74.01; H, 4.33; N, 8.71; S, 6.77.

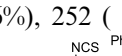
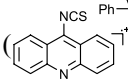
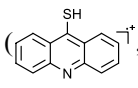
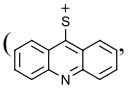
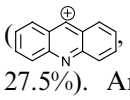
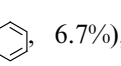
4.2.14. *N*-(4-Phenyl-3-(4′-chloro phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4n). Solvent of elution, $CHCl_3$/EtOAc (1:1); Yellow solid (0.210 g, 50%); mp 105–107 °C; IR (KBr) ν_{max}: 1616, 1533, 1482, 1288, 1085 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 4.01 (s, 3H, OCH_3), 6.51 (s, 1H, >C=CH–), 7.11–7.18 (q, 1H, Ar), 7.23–7.37 (m, 6H, Ar), 7.48–7.60 (m, 4H, Ar), 7.65–7.68 (d, 2H, Ar), 7.75–7.80 (t, 1H, Ar), 8.03–8.13 (dd, 2H, Ar); EI-MS: 493 (M^+, 10.9%), 492

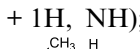
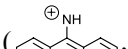
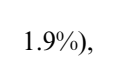
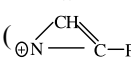
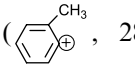
(M^+-H, 8.0%), 214 ( , 18.2%), 134 ( , 4.4%), 113 ( , 5.8%), 111 (, 29.1%). Anal. Calcd for $C_{29}H_{20}N_3SOCl$: C, 70.51; H, 4.05; N, 8.51; S, 6.49. Found: C, 70.60; H, 4.27; N, 8.45; S, 6.64.

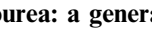
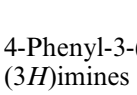
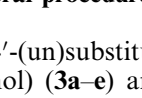
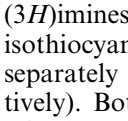
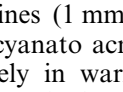
4.2.15. *N*-(4-Phenyl-3-(2′,3′-dimethyl phenyl)thiazol-2(3*H*)-ylidene)-4-methoxy acridin-9-amine (4o). Solvent of elution, $CHCl_3$/EtOAc (4:1); Orange solid (0.240 g, 60%); mp 150–153 °C; IR (KBr) ν_{max}: 1616, 1523, 1473, 1280, 1067 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.22–2.25 (2s, 6H, $CH_3 + CH_3$), 4.05 (s, 3H, OCH_3), 6.68 (s, 1H, >C=CH–), 7.20–7.34 (m, 8H, Ar), 7.45–7.60 (m, 4H, Ar), 7.90 (t, 1H, Ar), 8.05–8.08 (d, 1H, Ar), 8.25–8.27 (d, 1H, Ar); FAB-MS: 488.1142 (MH^+, 100%). Anal. Calcd for $C_{31}H_{25}N_3SO$: C, 76.38; H, 5.13; N, 8.62; S, 6.57. Found: C, 76.20; H, 5.23; N, 8.42; S, 6.62.

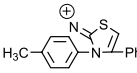
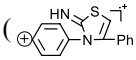
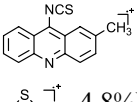
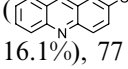
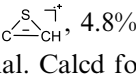
4.3. 1-[(2,4-(Un)substituted acridin-9-yl)-3-(4-phenyl-3-(2′,3′,4′(un)substituted phenyl)thiazol-2(3*H*)-ylidene)] isothiourea: a general procedure for synthesis of 5a–h

4-Phenyl-3-(2′,3′,4′-(un)substituted phenyl)thiazol-2-(3*H*)imines (1 mmol) (**3a**–**e**) and 2,4-(un)substituted 9-isothiocyanato acridine (1 mmol) (**2a**–**d**) were dissolved separately in warm THF (~10 and ~50 mL, respectively). Both the solutions were cooled to rt and then mixed together and allowed to stand at rt for one to four days. After completion of reaction (TLC) THF was allowed to evaporate at rt and the residue was subjected to column chromatography to give pure condensed product (**5a**–**h**).

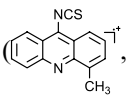
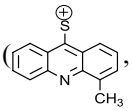
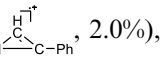
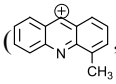
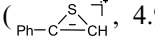
4.3.1. 1-[(Acridin-9-yl)-3-(3,4-diphenyl)thiazol-2(3*H*)-ylidene]isothiourea (5a). Solvent of elution, hexane/$CHCl_3$ (1: 4); Red solid (0.170 g, 40%); mp 180–182 °C; IR (KBr) ν_{max}: 3300, 1635, 1557, 1495, 1215, 1167 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.17–7.63 (m, 17H, Ar), 8.33–8.37 (d, 2H, Ar), 11.32 (s, 1H, NH exch.); EI-MS does not give M^+ ion peak, 430 (M^+-SCN, 1.9%), 429 (M^+-HSCN, 9.6%), 252 ( , 16.5%), 251 ( , 23.3%), 236 ( , 31.9%), 211 ( , 100%), 210 ( , 12.7%), 178 ( , 6.7%), 134 ( , 4.4%), 77 ($C_6H_5^+$, 27.5%). Anal. Calcd for $C_{29}H_{20}N_4S_2$: C, 71.31; H, 4.09, N, 11.47; S, 13.11. Found: C, 71.47; H, 4.35; N, 11.22; S, 13.22.

4.3.2. 1-[(Acridin-9-yl)-3-(4-phenyl-3-(2′-methyl phenyl) thiazol-2(3*H*)-ylidene)]isothiourea (5b). Solvent of elution, $CHCl_3$; Orange solid (0.155 g, 30%); mp 183–185 °C; IR (KBr) ν_{max}: 3353, 1620, 1555, 1454, 1217 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.91 (s, 3H, $-CH_3$), 6.06 (d, 2H, Ar), 6.17 (s, 1H, >C=CH–), 6.58–8.16 (m, 16H, 15H, Ar + 1H, NH); EI-MS does not give M^+ ion peak 207 ( , 1.3%), 193 ( , 1.9%), 134 ( , 10.3%), 116 ( , 1.8%), 102 (Ph–CH≡CH, 0.9%), 91 ( , 28.5%). Anal. Calcd for $C_{30}H_{22}N_4S_2$: C, 71.71; H, 4.38; N, 11.15; S, 12.75. Found: C, 72.07; H, 4.37; N, 11.34; S, 12.49.

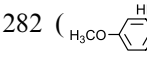
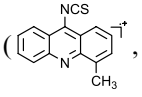
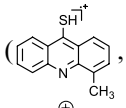
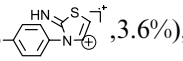
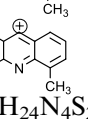
4.3.3. 1-[(2-Methyl acridin-9-yl)-3-(3,4-diphenyl)thiazol-2(3*H*)-ylidene]isothiourea (5c). Solvent of crystallization, THF; Yellow solid (0.175 g, 35%); mp 233–235 °C; IR (KBr) ν_{max}: 3410, 1622, 1496, 1445, 1280, 1176 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.55 (s, 3H, CH_3), 7.10–7.40 (m, 8H, Ar), 7.40–7.50 (m, 6H, Ar), 7.50–7.60 (t, 1H, Ar); 7.80–8.05 (m, 3H, Ar), 9.90 (br s, 1H, NH exch.); EI-MS do not give M^+ ion peak 252 ( , 50.8%), 251 ( , 84.8%), 250 ( , 100%), 225 ( , 3.65%), 192 ( , 8.0%), 134 ( , 5.0%), 77 ($C_6H_5^+$, 19.0%). Anal. Calcd for $C_{30}H_{22}N_4S_2$: C, 71.71; H, 4.38; N, 11.15; S, 12.75. Found: C, 71.99; H, 4.48; N, 11.24; S, 12.84.

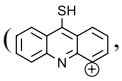
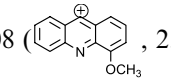
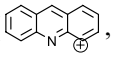
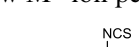
4.3.4. 1-[(2-Methyl acridin-9-yl)-3-(4-phenyl-3-(4′-methyl phenyl)thiazol-2(3*H*)-ylidene)]isothiourea (5d). Solvent of elution, $CHCl_3$/Pet. ether (4:1); Yellow solid (0.180 g, 35%); mp 180–182 °C; IR (KBr) ν_{max}: 3439, 2964, 1628, 1469, 1376, 1282, 1174, 1218 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.11 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 5.98–6.13 (d, 3H, Ar), 6.58 (s, 1H, >C=CH–), 6.88–6.92 (d, 2H, Ar), 7.08–7.16 (m, 3H, Ar), 7.36–7.49 (t, 1H, Ar), 7.50–7.54 (d, 1H, Ar), 7.60–7.65 (t, 1H, Ar), 7.77 (s, 1H, Ar), 7.97–8.15 (m, 4H, Ar); EI-MS does not give M^+ ion peak 266 ( , 56.5%), 265 ( , 77.6%), 251 ( , 18.2%), 250 ( , 100%), 192 ( , 9.8%), 134 (, 4.8%), 91 ( , 16.1%), 77 ($C_6H_5^+$, 5.8%). Anal. Calcd for $C_{31}H_{24}N_4S_2$: C, 72.09; H, 4.65; N, 10.85; S, 12.40. Found: C, 72.05; H, 4.54; N, 10.74; S, 12.42.

4.3.5. 1-[(4-Methyl acridin-9-yl)-3-(3,4-diphenyl)thiazol-2(3*H*)-ylidene]isothiourea (5e). Solvent of elution, Pet. ether/EtOAc (4:1); Yellow solid (0.150 g, 30%); mp 195–197 °C; IR (KBr) ν_{max}: 3449, 1594, 1496, 1445, 1280, 1176 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.90 (s, 3H, $-CH_3$), 6.04 (d, 1H, Ar), 6.34 (d, 1H, Ar), 6.64 (s, 1H, >C=CH–), 6.89 (d, 2H, Ar), 7.00–7.15 (m, 4H, Ar), 7.20–7.30 (m, 2H, Ar), 7.30–7.40 (m, 1H,

Ar), 7.40–7.50 (d, 1H, Ar), 7.50–7.60 (d, 1H, Ar), 7.65–7.75 (d, 1H, Ar), 7.75–7.85 (d, 1H, Ar), 8.05–8.15 (d, 1H, Ar), 8.15–8.25 (d, 1H, Ar), 10.15 (s, 1H, NH, exch.); EI-MS does not show M^+ ion peak but gave following fragment peaks. *m/z* 250 ( , 100%), 224 ( , 2.9%), 193 ( , 2.0%), 192 ( , 6.7%), 134 ( , 4.9%), 77 ($C_6H_5^+$, 20.9%). Anal. Calcd for $C_{30}H_{22}N_4S_2$: C, 71.71; H, 4.38; N, 11.15; S, 12.74. Found: C, 71.67; H, 4.01; N, 11.09; S, 12.41.

4.3.6. 1-[(4-Methyl acridin-9-yl)-3-(4-phenyl-3-(4′-methyl phenyl)thiazol-2(3*H*)-ylidene)]isothiourea (5f). Solvent of elution, $CHCl_3$/Pet. ether (4:1); Orange solid (0.180 g, 35%); mp 175–177 °C; IR (KBr) ν_{max}: 3437, 1618, 1464, 1376, 1280, 1174, 1218 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.10 (s, 3H, CH_3), 2.94 (s, 3H, CH_3), 5.93–6.07 (d, 3H, Ar), 6.57(s,1H, >C=CH–), 7.01–8.19 (m, 13H, Ar); FAB-MS: *m/z* 518.09 (MH^+, 11.5%), Anal. Calcd for $C_{31}H_{24}N_4S_2$: C, 72.09; H, 4.65; N, 10.85; S, 12.40. Found: C, 72.24; H, 4.69; N, 10.84; S, 12.78.

4.3.7. 1-[(4-Methyl acridin-9-yl)-3-(4-phenyl-3-(4′-methoxyphenyl)thiazol-2(3*H*)-ylidene)]isothiourea (5g). Solvent of elution, Pet. ether/EtOAc (4:1); Orange solid (0.212 g, 40%); mp 195–197 °C; IR (KBr) ν_{max}: 3459, 1607, 1508, 1464, 1278, 1170, 1214 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.89 (s, 3H, CH_3), 3.72 (s, 3H, OCH3), 5.83–5.91 (d, 2H, Ar), 6.60 (s, 1H, >C=CH–), 6.80–6.90 (d, 2H, Ar), 7.00–7.10 (m, 3H, Ar), 7.30–7.50 (m, 3H, Ar), 7.50–7.60 (d, 1H, Ar), 7.60–7.70 (t, 2H, Ar), 7.81–7.89 (d, 1H, Ar), 7.99–8.02 (d, 1H, Ar), 8.13–8.16 (d, 1H, Ar), 9.83 (s, 1H, NH exch.); EI-MS does not show M^+ ion peak but show other fragments 282 ( , 38.6%), 281 (*m/z* 282 –H, 42.10%), 252 (*m/z* 282 –HCHO, 6.2%), 251 (*m/z* 282 –OCH_3 ,17.4%), 250 ( , 100%), 210 ( , 24.3%), 205 ( ,3.6%), 192 ( , 6.0%), 77 ($C_6H_5^+$, 8.6%). Anal. Calcd for $C_{31}H_{24}N_4S_2O$: C, 69.92; H, 4.51; N, 10.52; S, 12.03. Found: C, 69.67; H, 4.54; N, 10.33; S, 12.12.

4.3.8. 1-[(4-Methoxy acridin-9-yl)-3-(3,4-diphenyl)thiazol-2(3*H*)-ylidene]isothiourea (5h). Solvent of elution, CCl_4/EtOAc (9:1); Yellow solid (0.182 g, 35%); mp 210–212 °C; IR (KBr) ν_{max}: 3458, 2944, 1626, 1477, 1378, 1274, 1217, 1075 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 4.10 (s, 3H, OCH_3), 7.10–7.40 (m, 11H, Ar), 7.65–7.73 (m, 2H, Ar), 7.77–7.84 (m, 2H, Ar), 7.91–7.96 (d, 2H, Ar), 8.18–8.24 (d, 1H, Ar), 11.30 (s, 1H, NH exch.); EI-MS, does not show M^+ ion peak but show other fragments, 251 ( , 1.5%), 210 ( , 100%), 208 ( , 2.7%), 178 ( , 1.2%), 134 ( , 1.9%), 77 ($C_6H_5^+$, 12.60%). Anal. Calcd for $C_{30}H_{22}N_4S_2O$: C, 69.49; H, 4.24; N, 10.81; S, 12.35. Found: C, 69.40; H, 4.16; N, 10.43; S, 12.23.

4.4. Anti-inflammatory activity

Anti-inflammatory activity screening[23] was carried out using carrageenin induced paw oedema in albino rats. Oedema in one of the hind paws was induced by injection of carrageenin solution (0.1 mL of 1%) into planter apponeurosis. The volume of the paw was measured plethysmographyically immediately after and 3 h after the injection of the irritant. The difference in volume gave the amount of oedema developed. Percent inhibition of the oedema between the control group and compound treated groups was calculated and compared with the group receiving a standard drug.

4.5. Analgesic activity

Analgesia was measured by the writhing assay[24] using Swiss mice (15–20 g). Female mice were screened for writhing on day one, by injecting intraperitonially 0.2 mL of a 0.02% aqueous solution of phenylquinone. They were kept on a flat surface and the number of writhes of each mouse was recorded for 20 min. The mice showing significant (>10) writhes were sorted out and used for analgesic assay on the following day. The mice consisting of 5 in each group and showing significant writhing were given orally a 50 mg/kg p.o. dose of the test compounds 15 min prior to phenylquinone challenge. Writhing was again recorded for each mouse in a group and a percentage protection was calculated using following formula:

$$\text{Protection} = 100 - [\{(\text{No. writhings for treated mice})/(\text{No. of writhings for untreated mice})\} \times 100]$$

This was taken percent analgesia response and was averaged in each group of mice. Percent of animals exhibiting analgesia was determined.

4.6. Kinase inhibition activity[25–27]

4.6.1. Biochemical reagents. Sodium orthovanadate, EGTA, EDTA, Mops, β-glycerophosphate, phenylphosphate, sodium fluoride (NaF), dithiothreitol (DTT), bovine serum albumin (BSA), nitrophenylphosphate, leupeptin, aprotinin, pepstatin, soybean trypsin inhibitor, benzamidine, histone H1(type III-S) were obtained from Sigma Chemicals. [γ-^{33}P]-ATP was obtained from Amersham. The GS-1 peptide (YRRAAVPPSPSLSRHSSPHQSpEDEEE) was synthesized by the Peptide Synthesis Unit, Institute of Biomolecular Sciences, University of Southampton, Southampton SO16 7PX, UK.

4.6.2. Buffers

4.6.2.1. Homogenization buffer. 60 mM β-glycerophosphate, 15 mM *p*-nitrophenyl-phosphate, 25 mM Mops (pH 7.2), 15 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM NaF, 1 mM phenylphosphate, 10 μg leupeptin/mL, 10 μg aprotinin/mL, 10 μg soybean trypsin inhibitor/mL and 100 μM benzamidine.

4.6.2.2. Buffer A. 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 μg heparin/mL.

4.6.2.3. Buffer C. Homogenization buffer, but 5 mM EGTA, no NaF and no protease inhibitors.

4.6.3. Kinase preparations and assays. Kinase activities were assayed in Buffer A or C, at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities calculated as pmoles of phosphate incorporated for a 10 min incubation. The activities were calculated in percentage of the maximal activity, in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethylsulfoxide.

4.6.4. GSK-3α/β. was either purified from porcine brain by affinity purification on an immobilized fragment of axin.[25] It was assayed, following a 1/100 dilution in 1 mg BSA/mL, 10 mM DTT, with 5 μL 40 μM GS-1 peptide as a substrate, in buffer A, in the presence of 15 μM [γ-^{33}P]-ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid/litre of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid.

4.6.5. CDK1/cyclin B. was extracted in homogenization buffer from M phase starfish (*Marthasterias glacialis*) oocytes and purified by affinity chromatography on $P9^{CKShs1}$ sepharose beads, from which it was eluted by free $P9^{CKShs1}$ as reported previously.[26,27] The kinase activity was assayed in buffer C, with 1 mg histone H 1/mL, in the presence of 15 μM [γ-^{32}P]-ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 10 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto P81 phosphocellulose papers and treated as described above.

4.6.6. CDK5/p25. was reconstituted by mixing equal amounts of recombinant mammalian CDK-5 and p25 expressed in *E. coli* as GST (Glutathione-S-transferase) fusion proteins and purified by affinity chromato-graphy on glutathione-agarose (vectors kindly provided by Dr. J. H. Wang) (p25 is a truncated version of p35, the 35KDa CDK5 activator). Its activity wasassayed in buffer C as described for CDK1/cyclinB.

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References and notes

1. (a) Bouffier, L.; Demeunynck, M.; Milet, A.; Dumy, P. *J. Org. Chem.* **2004**, *69*, 8144; (b) Chiron, J.; Galy, J.-P. *Synlett* **2004**, 392; (c) Chiron, J.; Galy, J.-P. *Synthesis* **2004**, 313.
2. (a) Chen, H. *Huaxue Yanjiu Yu Yingyong* **2000**, *12*, 164; (b) Sivan, S.; Tuchman, S.; Lotan, N. *BioSystems* **2003**, *70*, 21.
3. Flock, S.; Bailly, F.; Bailly, C.; Waring, M. J.; Henichart, J.-P.; Colson, P.; Houssier, C. *J. Biomol. Struct. Dyn.* **1994**, *11*, 881.
4. (a) Gooch, B. D.; Beal, P. A. *J. Am. Chem. Soc.* **2004**, *126*, 10603; (b) Stefanska, B.; Bontemps-Gracz, M. M.; Antonini, I.; Martelli, S.; Arciemiuk, M.; Piwkowska, A.; Rogacka, D.; Borowski, E. *Bioorg. Med. Chem.* **2005**, *13*, 1969.
5. Demeunynck, M. *Expert Opin. Ther. Patents* **2004**, *14*, 55.
6. Antonini, I.; Polucci, P.; Magnano, A.; Sparapani, S.; Martelli, S. *J. Med. Chem.* **2004**, *47*, 5244.
7. For review: Martinez, R.; Chacon-Garcia, L. *Curr. Med. Chem.* **2005**, *12*, 27.
8. (a) Henry, D. W. *Chem. Heterocycl. Compd.* **1973**, *9*, 829; (b) Wolfe, A. D. *Antibiotics* **1975**, *3*, 203.
9. (a) Chen, Y. L.; Lu, C. M.; Chen, I. L.; Tsao, L. T.; Wang, J. P. *J. Med. Chem.* **2002**, *45*, 4689; (b) Chen, Y. L.; Chen, I. L.; Lu, C. M.; Treng, C. C.; Tsao, L. T.; Wang, J. P. *Bioorg. Med. Chem.* **2003**, *11*, 3921.
10. Skotnicki, J. S.; Gilman, S. C. U S US 851536; *Chem. Abstr.* 112, **1990**, 118672e.
11. (a) Meijer, L. *Chem. Biol.* **2003**, *10*, 1255; (b) Meijer, L. *Acc. Chem. Res.* **2003**, *36*, 417; (c) Meijer, L.; Flajolet, M.; Greengard, P. *Trends Pharmacol. Sci.* **2004**, *25*, 471.
12. Stein, C. A.; Mori, K.; Loke, S. L.; Subasinghe, C.; Shinozuka, K.; Cohen, J. S.; Neckers, L. M. *Gene* **1988**, *72*, 333.
13. Sebestik, J.; Matejka, P.; Hlavacek, J.; Stibor, I. *Tetrahedron Lett.* **2004**, *45*, 1203.
14. Di Giorgio, C.; Delmas, F.; Filloux, N.; Robin, M.; Seferian, L.; Azas, N.; Gasquet, M.; Costa, M.; Timon-David, P.; Galy, J.-P. *Antimicrob. Agents Chemother.* **2003**, *47*, 174.
15. (a) Shridhar, D. R.; Sastry, C. V. R.; Lal, K.; Bansal, O. P.; Sondhi, S. M. Gazette of India, March 28, 1987; p 213, INDIAN Patent no. 159118; (b) Sondhi, S. M.; Verma, R. P.; Singhal, N.; Shukla, R.; Raghubir, R.; Dubey, M. P. *Indian Drugs* **1999**, *36*, 50; (c) Sondhi, S. M.; Sharma, V. K.; Singhal, N.; Verma, R. P.; Shukla, R.; Raghubir, R.; Dubey, M. P. *Phosphorus Sulphur Silicon* **2000**, *156*, 21; (d) Sondhi, S. M.; Johar, M.; Singhal, N.; Dastidar, S. G.; Shukla, R.; Raghubir, R. *Monatsh. Chem.* **2000**, *131*, 511; (e) Sondhi, S. M.; Johar, M.; Rajvanshi, S.; Dastidar, S. G.; Shukla, R.; Raghubir, R.; Lown, J. W. *Aust. J. Chem.*

2001, *54*, 69; (f) Sondhi, S. M.; Rajvanshi, S.; Johar, M.; Bharti, N.; Azam, A.; Singh, A. K. *Eur. J. Med. Chem.* **2002**, *37*, 835; (g) Sondhi, S. M.; Goyal, R. N.; Lahoti, A. M.; Singh, N.; Shukla, R.; Raghubir, R. *Bioorg. Med. Chem.* **2005**, *13*, 3185.

16. For review: (a) Sondhi, S. M.; Singhal, N.; Johar, M.; Reddy, B. S. N.; Lown, J. W. *Curr. Med. Chem.* **2002**, *9*, 1045; (b) Sondhi, S. M.; Rajvanshi, S.; Singh, N.; Jain, S.; Lahoti, A. M. *Cent. Eur. J. Chem.* **2004**, *2*, 141.
17. Albert, A.; Ritiche, B. *Org. Synth.* **1960**, *3*, 53.
18. Allen, C. F. H.; McKbee, G. H. W. *Org. Synth.* **1959**, *2*, 15.
19. Mahajan, M. P.; Vaudeva, S. K.; Ralhan, N. K. *Indian J. Chem.* **1972**, *10*, 318.
20. Vlassa, M.; Kezdi, M.; Bogdan, M. *J. Prakt. Chem.* **1985**, *327*, 1007.
21. Sondhi, S. M.; Bhattacharjee, G.; Jameel, R. K.; Shukla, R.; Raghubir, R.; Lozach, O.; Meijer, L. *Cent. Eur. J. Chem.* **2004**, *2*, 1.
22. For review: Denny, W. A. *Curr. Med. Chem.* **2002**, *9*, 1655.
23. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
24. Singh, P. P.; Junnarkar, A. Y.; Rao, C. S.; Verma, R. K.; Shridhar, D. R. *Methods Find. Expt. Clin. Pharmacol.* **1983**, *5*, 601.
25. Primot, A.; Baratte, B.; Gompel, M.; Borgne, A.; Liabeuf, S.; Romette, J. L.; Costantini, F.; Meijer, L. *Protein Expr. Purif.* **2000**, *20*, 394.
26. Leclerc, S.; Garnier, M.; Hoessel, R.; Marko; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Biernat, J.; Mandelkow, E.-M.; Eisenbrand, G.; Meijer, L. *J. Biol. Chem.* **2001**, *276*, 251.
27. Leost, M.; Schultz, C.; Link, A.; Wu, Y.-Z.; Biernat, J.; Mandelkow, E.-M.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Zaharevitz, D. W.; Gussio, R.; Senderovitz, A.; Sausville, E. A.; Kunick, C.; Meijer, L. *Eur. J. Biochem.* **2000**, *267*, 5983.