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Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases

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Abstract—A series of *N*-(acridin-9-yl)-4-(benzo[*d*]imidazol/oxazol-2-yl) benzamides has been synthesized by the condensation of 9-aminoacridine derivatives with benzimidazole or benzoxazole derivatives. Condensation of 2-hydroxy naphthaldehyde with functionalized diamines leads to the formation of Schiff's bases and not imidazole derivatives. All these compounds were characterized by correct FT-IR, ^{1}H NMR, MS and elemental analyses. These compounds were screened for anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activities. Compounds **11** and **7e(f)** showed good anti-inflammatory (35.8% at 50 mg/kg po) activity and good analgesic activity (60% at 50 mg/kg po), respectively. Compound **3b** showed significant in vitro activity against CDK-5 (IC_{50} = 4.6 μM) and CDK-1(IC_{50} = 7.4 μM) and compound **3a** showed moderate CDK-5 inhibitory activity (IC_{50} = 7.5 μM). The other compounds showed moderate anti-inflammatory and analgesic activities.

1. Introduction

Benzimidazole[1–4] and benzoxazole[5–7] derivatives possessing anti-inflammatory, analgesic and kinase inhibition activities[8–10] have been reported in some literature. Apart from the above-mentioned activities some interesting efficacy of antiallergic,[11] antiproliferative,[12,13] antitumour,[14] antiHIV,[15] and antibacterial[16] activities on benzimidazole derivatives has been reported. Antituberculosis[17] activities of benzoxazole derivatives were also reported in the literature.

Due to broad spectrum of activities reported in the literature so far, we have synthesized a number of benzimidazole and benzoxazole derivatives targeting for potent molecules possessing anti-inflammatory, analgesic[18–20] and kinase inhibitory activities.[21] We will describe in this paper the synthesis, screening results of in vivo anti-inflammatory and analgesic activities and in vitro kinase inhibitory activities.

Keywords: Benzimidazole derivatives; Benzoxazole derivatives; Schiff's bases; Anti-inflammatory; Analgesic; CDK-1; CDK-5 and GSK-3.
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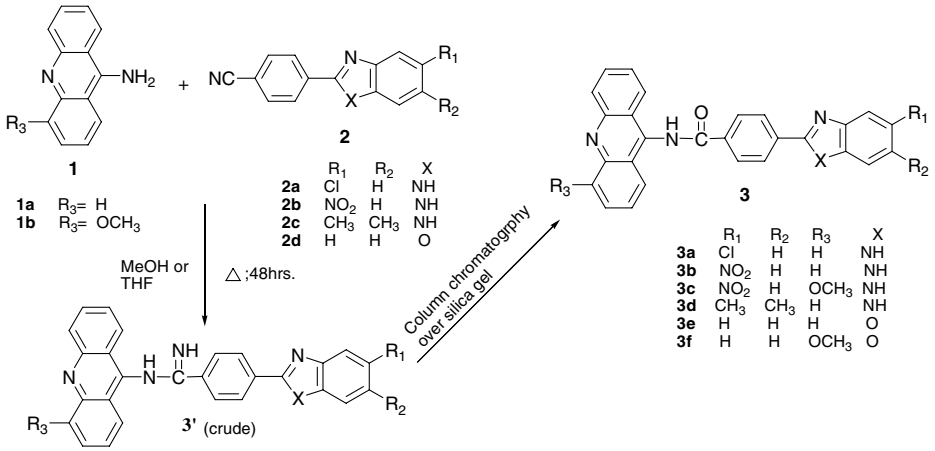
2. Results and discussion

2.1. Chemistry

N-(Acridin-9-yl)-4-(benzo[*d*]imidazol-2-yl)benzamide derivatives **3a–d** (Scheme 1) have been synthesized by the condensation of benzimidazole derivatives **2a–c** with 9-aminoacridine derivatives **1a–b** and *N*-(acridin-9-yl)-4-(benzo[*d*]oxazol-2-yl)benzamide derivatives **3e–f** (Scheme 1) were synthesized by the reaction of benzoxazole derivative **2d** with 9-aminoacridine derivatives **1a–b** by refluxing in methanol or in THF (tetrahydrofuran) for 48 h. The condensed products **3a–d** were purified by chromatography on a silica gel column. The precursor benzimidazole derivatives **2a–c** were synthesized by the condensation of 4-cyanobenzaldehyde with *o*-phenylenediamines, and benzoxazole derivative **2d** was synthesized by the reaction of 4-cyanobenzaldehyde with *o*-amino phenol by refluxing in nitrobenzene.[22] The key intermediates, 9-aminoacridines **1a–b**, were synthesized by following the reaction procedure reported in the literature.[23] Spectral data and elemental analyses of compounds **3a–f** fully support the structures assigned to them.

It is interesting to note that condensation of 9-aminoacridine derivative **1** (Scheme 1) with benzimidazole or



Scheme 1.

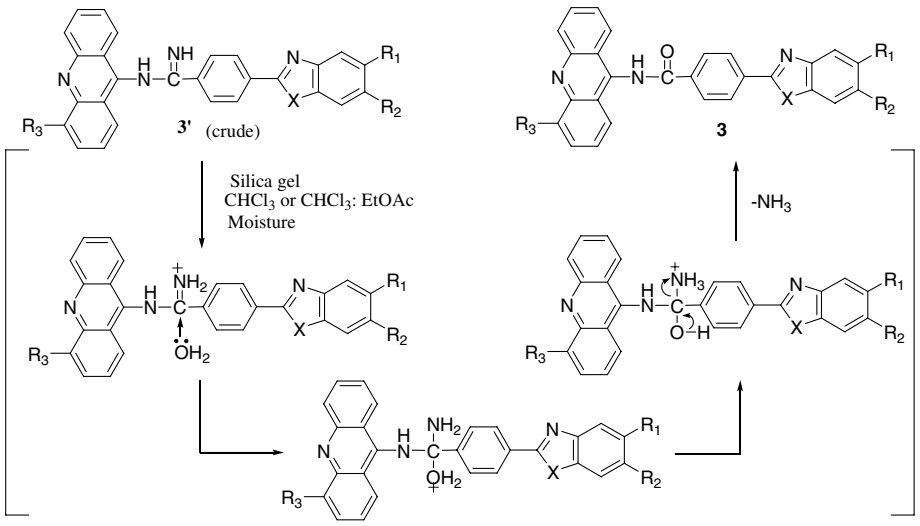
benzoxazole derivative **2** should give condensation product **3′**. But we got a hydrolysed product **3** instead of getting **3′** (Scheme 1).

The formation of **3** can arise from hydrolysis of **3′** during purification by chromatography on a silica gel column. A probable mechanism for the formation of **3** from **3′** is suggested in Scheme 2. Similar type of observation is also reported in the literature.[24] FT-IR spectra of compound **3a** showed amide stretching at 1707 (NHCO) and FAB-MS of **3a** shows MH^+ ion peak at *m/z* 449 (MH^+; 4%) Calcd for $C_{27}H_{17}N_4OCl$ *m/z* 448 and the fragmented cations observed in the mass spectra fully support the structure of **3a** (Scheme 2).

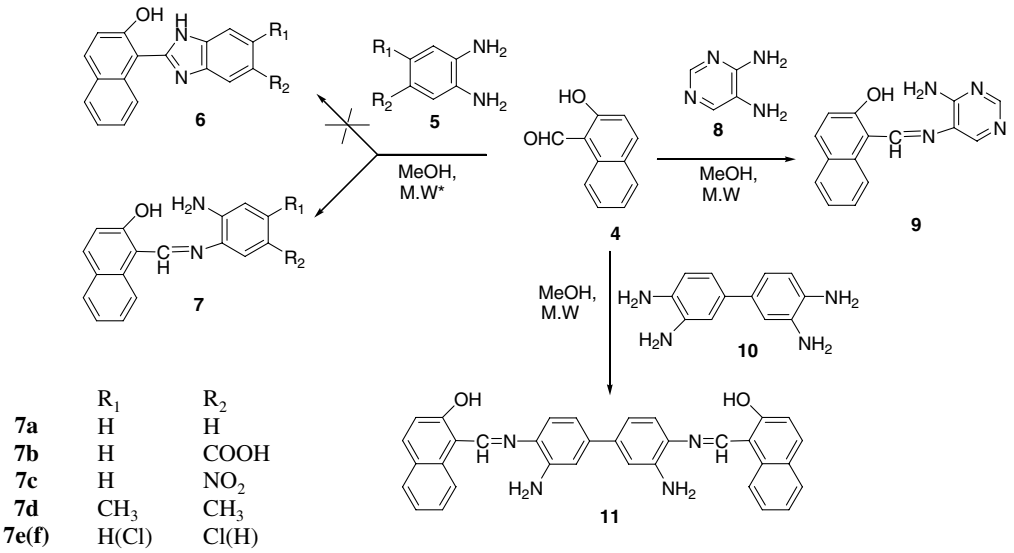
In our earlier studies of this research, the reactions of *p*-cyanobenzaldehyde on condensation with *o*-phenylenediamines gave benzimidazole derivatives. We tried to extend this reaction by using 2-hydroxynaphthaldehyde and various *o*-phenylenediamines and 2,3-diamino pyrimidines. 2-Hydroxy naphthaldehyde (**4**) was synthesized from 2-hydroxy naphthol by following the procedure reported in the literature.[25] 2-Hydroxy naphthaldehyde (**4**) and (un) substituted *o*-phenylenediamine (**5**) were taken in equimolar ratio and then heated at 140–150 °C in nitrobenzene for 24 h. Solvent was removed under reduced pressure and the residue left behind was crystallized from THF to give pure condensed product **7a** without affording compound **6** (Scheme 3). The same reaction was also achieved by using microwave irradiation as mentioned below.

2-Hydroxy naphthaldehyde (**4**) and *o*-phenylenediamine (**5**) were dissolved in methanol in equimolar ratio and then the mixture irradiated in microwave oven for 30 min at 100 watt. During irradiation, the compound separated out and after irradiation the solvent was removed under reduced pressure and the crude product was crystallized from THF to give pure condensed product **7a** (Scheme 3). The product **7a** (Scheme 3) obtained by refluxing in nitrobenzene and by microwave irradiation method was found to be identical (from m.p., mix m.p.; TLC and co-TLC).

All other substituted *o*-phenylenediamines, that is, **5b–e**, 4,5-diamino pyrimidine (**8**) and 3,3′-diaminobenzimidine



Scheme 2.



	R_1	R_2
7a	H	H
7b	H	COOH
7c	H	NO_2
7d	CH_3	CH_3
7e(f)	H(Cl)	Cl(H)

Scheme 3.

(**10**) were condensed with 2-hydroxy naphthaldehyde (**4**) by microwave irradiation to give compounds **7b–e(f)**, **9** and **11** (Scheme 3).

It is interesting to note that the reaction of **5b** and **5c** condensed with aldehyde **4** gave only regioisomers **7b** and **7c**, respectively. This is due to a more basic amino group in meta position to –COOH and NO_2. Spectral data of **7b** and **7c** fully support the structures assigned to them. In case of **7d**, only one product is possible due to symmetrical structure of **5d**.

4-Chloro-*o*-phenylenediamine on condensation with 2-hydroxy naphthaldehyde gave a mixture of two isomeric products **7e** and **7f** in the approximate ratio of 4:1. The ratio can be explained on the basis of inductive and mesomeric effect of the chloro group. ^{1}H NMR (300 MHz; DMSO-d_6) of **7e(f)** shows doubling of peaks for –NH_2– and –CH=N– at δ 5.26 and 5.43 and δ 9.61–9.65, respectively, indicating that condensation product is a mixture of **7e** and **7f**. FAB-MS of **7e(f)** shows a MH^+ ion peak at *m*/*z* 297.92 (35%). Spectral data of **7e(f)** fully support the structure assigned to it.

In diamino pyrimidine (**8**), –NH_2 group in meta position to both the ring nitrogens is more basic as compared to the other –NH_2 group and reacted with the –CHO group to give condensation product **9**. Similar observations are already reported in the literature.[26]

2.2. Biological activity

The acridinyl-benzimidazole **3a–d**, acridinyl-benzoxazole derivatives **3e–f** and Schiff's bases **7a–e(f)** and **11** were tested for: (i) anti-inflammatory activity in the carrageenin-induced paw oedema model in albino rats at 50 mg/kg po, according to the method of Winter et al.[27] (ii) analgesic activity[28] in the phenylquinone writhing assay at 50 mg/kg po and (iii) cyclin-dependent kinase (CDK-1 and CDK-5) and glycogen synthase kinase (GSK-3) inhibitory activity[29–31] and the results are summarized in Table 1.

In summary, activity data show that compound **11** exhibited good anti-inflammatory and compound **7e(f)** exhibited good analgesic activity, whereas compound **3b** (R_1 = NO_2, R_2 = H, R_3 = H and X = NH) showed

Table 1. Anti-inflammatory, analgesic and kinase inhibition activities of compounds **3a–f**, **7a–d**, **e(f)** and **11**

Compound	Anti-inflammatory activity (%) 50 mg/kg po	Analgesic activity (%) 50 mg/kg po	Kinase IC_{50} (μM)		
			CDK-1	CDK-5	GSK-3
3a	0.0	25	15	**7.5**	>100
3b	3.5	25	**7.4**	**4.6**	42
3c	7.5	0.0	—	—	—
3d	0.0	25	>10	>10	>10
3e	0.0	25	>10	>10	>10
3f	0.0	25	>10	>10	>10
7a	26.6	30	>10	—	>10
7b	25.5	20	>10	—	>10
7c	28.2	40	>10	—	>10
7d	28.2	30	>10	—	>10
7e(f)	31.4	**60**	>10	—	>10
11	**35.8**	50	>10	—	>10
Ibuprofen	38.8	50			

significant activity against CDK-5, while compounds **3a** and **3b** showed moderate activity against CDK-5 and CDK-1, respectively.

Data reported in Table 1 suggest that compound *N*-(acridin-9yl)-4-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl) (**3b**) shows significant in vitro kinase inhibitory activity against CDK-5 (IC_{50} 4.6 μM) and CDK-1 (IC_{50} = 7.4 μM), whereas compound **3a** has some activity against CDK-5 (IC_{50} = 7.6 μM). Compounds **3d–f** do not show any activity towards CDKs and GSK. These observations revealed that the nature of the substituents at R_1 position of benzimidazole ring of acridinyl-benzimidazole derivative has a major impact on CDK inhibitory activity. The structures of the active compounds indicate that substitution at R_1 = Cl for **3a** and R_1 = NO_2 for **3b** might enhance potency of hydrogen bonding/acceptor/donor in benzimidazoles **3a** and **3b** which probably favours interaction with the active site of the CDK enzymes, whereas other analogues with R_1 = CH_3 and/or H lost their potency.

In case of Schiff's bases, compound 4,4′bis [1-{(2-aminophenylimino) methyl] naphthalen-2-ol (**11**) showed good anti-inflammatory activity 35.8% at 50 mg/kg po, whereas other derivatives showed moderate anti-inflammatory activity. The activity in case of **11** increases because of bis nature which is favourable for anti-inflammatory activity, whereas compound [(2-aminophenylimino) methyl] naphthalen-2-ol (**7a**) showed significant anti-inflammatory activity. Compounds **7e(f)** also showed good anti-inflammatory, probably due to enhanced activity by the presence of the Cl group. Compounds **7e(f)** and **11** showed good analgesic activity. Similarly ananlgesic activity of **11** (bis form of **7a**) was increased, as compared to **7a**.

3. Conclusion

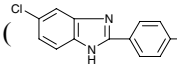
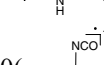
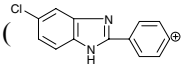
In conclusion, according to the results of screening tests, the bioactivity of the described compounds shows a clearcut relation with substitution on R_1. This structure activity relationship observation will be helpful to design novel analogues of this class of compounds as prospective kinase inhibitors and anti-inflammatory agents.

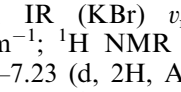
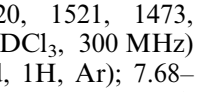
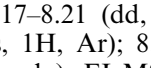
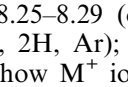
4. Experimental

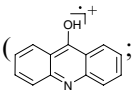
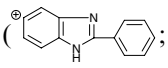
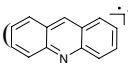
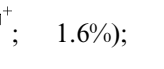
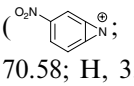
Melting points (mp) were determined on a JSGW apparatus and are uncorrected. Domestic microwave oven model M197 DL (SAMSUNG) was used for microwave irradiation. IR spectra were recorded using a Perkin Elmer 1600FT Spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker WH-300 Spectrometer in a ca. 5–15% (w/v) solution in DMSO-d_6 (TMS as internal standard). The Mass spectrometer peak measurements were made by comparison with perfluorotributylamine using AEI MS-9 double focusing high-resolution mass spectrometer at a resolving power of 15,000. FAB-MS was reordered on Jeol SX-120 (FAB) spectrometer. Electron impact (EI) MS were recorded on a Jeol D-300 mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapour or by irradiation with ultraviolet light (254 nm). Column chromatography was performed by using Qualigens silica gel for column chromatography (60–120 mesh). 2% Gum acacia was used as a vehicle for oral administration of the test compounds.

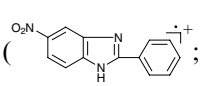
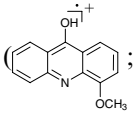
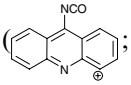
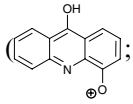
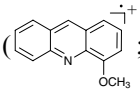
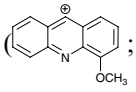
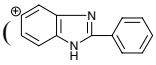
4.1. General procedure for synthesis of 3

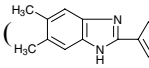
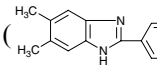
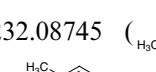
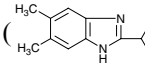
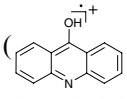
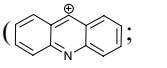
4.1.1. Synthesis of *N*-(acridin-9-yl)-4-(5-chloro-1*H*-benzo[*d*]imidazol-2yl)benzamide (3a). 4-(5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)benzonitrile (**2a**) (253 mg; 1 mmol) was dissolved in MeOH (30 mL) and to it was added 9-aminoacridine (**1a**) (194 mg; 1 mmol). The reaction contents were heated under reflux for 48 h and then the solvent was removed under reduced pressure. The solid residue left behind was dissolved in methanol and to this solution was added silica gel (∼20 g) and then the solvent was removed under reduced pressure to give a crude product absorbed over silica gel. Column was packed with silica gel using pet. ether as solvent. Crude product adsorbed over silica gel was packed on the top of silica gel column and then elution was done with pet. ether, Chloroform/pet. ether (4:1) to remove side products. Further elution with chloroform gave pure condensed product **3a**. Similarly compounds **3b–d** were synthesized.

Solvent of elution, $CHCl_3$; white solid (0.300 g, 50%); mp 215 (d) °C; IR (KBr) ν_{max}: 3409, 3279, 1707, 1608, 1545, 1482, 1439, 1297 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.20–7.30 (m, 4H, 3H Ar + 1H, NH, exch.); 7.55 (d, 2H, Ar); 7.60 (m, 1H, Ar); 7.75 (m, 3H, Ar); 8.05 (dd, 2H, Ar); 8.25 (dd, 2H, Ar); 8.35 (d, 2H, Ar); 11.75 (s, 1H, NH exch.). FAB MS: *m*/*z* 449 (MH^+, 4%); 255 ( ; 35%); 254 (*m*/*z* 255; 100.0%); 228 ( ; 2.0%); 227 ( ;12%); 220(; 2.0%). Anal. Calcd for $C_{27}H_{17}N_4OCl$: C, 72.24; H, 3.79; N, 12.48. Found: C, 72.00; H, 3.82; N, 12.57.

4.1.2. Synthesis of *N*-(acridin-9-yl)-4-(5-nitro-1*H*-benzo[*d*]imidazol-2yl)benzamide (3b). Solvent of elution, pet. ether/EtOAc (4:1); white solid (0.300 g, 50%); mp 215 °C; IR (KBr) ν_{max}: 3294, 1620, 1521, 1473, 1339 cm^{-1}; ^{1}H NMR (DMSO-d_6 + $CDCl_3$, 300 MHz) δ: 7.20–7.23 (d, 2H, Ar); 7.58–7.59 (d, 1H, Ar); 7.68–7.73 (q, 2H, Ar); 7.84–7.86 (d, 4H, Ar); 7.93–7.96 (d, 1H, Ar); 8.17–8.21 (dd, 1H, Ar); 8.25–8.29 (dd, 1H, Ar); 8.32 (s, 1H, Ar); 8.39–8.42 (d, 2H, Ar); 8.57 (s, 1H –NH–, exch.). EI MS, do not show M^+ ion peak. 281 ( 1.2%); 239 ( ; 2.8%); 221 ( ;1.2%); 220 ( ; 1.2%);

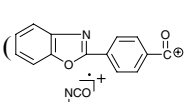
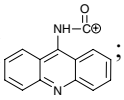
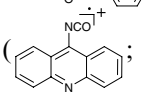
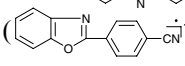
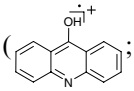
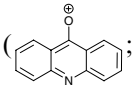
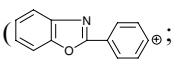
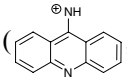
195 ( ; 7.4%); 193 ( ; 2.2%);179 ( ; 1.6%); 136 ( ; 28%);135 ( ; 4.5%). Anal. Calcd for $C_{27}H_{17}N_5O_3$: C, 70.58; H, 3.70; N, 15.25. Found: C, 70.72; H, 3.83; N, 15.00.

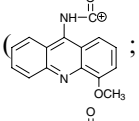
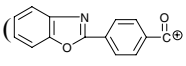
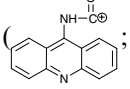
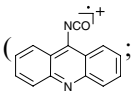
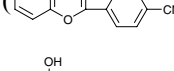
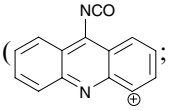
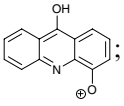
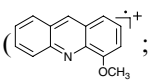
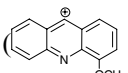
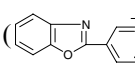
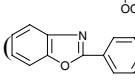
4.1.3. Synthesis of *N*-(4-methoxy acridin-9-yl)-4-(5-nitro-1*H*-benzo[*d*]imidazol-2yl)benzamide (3c). Solvent of elution, pet. ether/EtOAc (1:1); white solid (0.300 g, 65%); mp >250 °C; IR (KBr) ν_{max}: 3294, 1625, 1585, 1522, 1072 cm^{-1}; ^1H NMR (DMSO-d_6 + $CDCl_3$, 300 MHz) δ: 4.08 (s, 3H, OCH_3); 7.13–7.24 (m, 3H, Ar); 7.55–7.66 (m, 1H, Ar); 7.70–7.73 (d, 1H, Ar); 7.87–7.93 (m, 4H, Ar); 8.14–8.18 (dd, 1H, Ar); 8.27–8.29 (d, 1H, Ar); 8.39–8.49 (d, 3H, Ar); 8.54 (s, 1H, NH exch.); 10.97 (s, 1H, NH exch.). EI MS (*m*/*z* relative intensity) 239 ( ; 3.0%); 225 ( ; 74.6%); 219 ( ; 0.6%); 210 ( ; 100%); 209 ( ; 4.7%); 208 ( ; 1.1%); 193 ( ; 1.0%). Anal. Calcd for $C_{28}H_{19}N_5O_4$: C, 68.71; H, 3.88; N, 14.31. Found: C, 68.93; H, 4.00; N, 14.62.

4.1.4. Synthesis of *N*-(acridin-9yl)-4-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2yl)benzamide (3d). Solvent of elution, $CHCl_3$/EtOAc (4:1); white solid (0.300 g, 62%); mp 285 °C; IR (KBr) ν_{max}: 3190, 1640, 1611, 1443, 1278 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.37 (s, 6H, CH_3 + CH_3); 7.27–7.32 (t, 2H, Ar); 7.45 (s, 2H, Ar); 7.57–7.60 (d, 2H, Ar); 7.75–7.80 (t, 2H, Ar); 8.02–8.05 (d, 2H, Ar); 8.26–8.35 (dd, 4H, Ar); 11.80 (s, 1H, NH exch.). HR MS (*m*/*z*; relative intensity). No M^+ ion peak. 249.10197 ( ; 2.32%); 247.11069 ( ; 100.00%); 246.10313 (247.11069-; 53.00%); 232.08745 ( ; 58.75%); 221.10695 ( ; 2.63%); 195.06837 ( ; 9.10%); 178.06426 ( ; 2.02%). Anal. Calcd for $C_{29}H_{22}N_4O$: C, 78.73; H, 4.97; N, 12.66. Found: C, 78.41; H, 5.01; N, 12.51.

4.1.5. Synthesis of *N*-(acridin-9-yl)-4-(benzo[*d*]oxazol-2-yl)benzamide (3e). 4-(Benzo[*d*]oxazol-2-yl)benzonitrile (**2d**) (220 mg; 1 mmol) was dissolved in MeOH (30 mL) and to it was added 9-aminoacridine (**1a**) (194 mg; 1 mmol). The reaction contents were heated under reflux for 48 h and then the solvent was removed under reduced pressure to give a crude product. This crude product was purified by column chromatography over silica gel. Elution with pet. ether/chloroform (1:1) removed the side product and further elution with pet. ether/chloroform (1:4) gave pure product **3e**.

Compound **3f** was similarly synthesized.

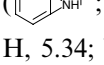
Solvent of elution, pet. ether/$CHCl_3$ (1:4); white solid (0.300 g, 62%); mp 175 °C; IR (KBr) ν_{max}: 3297, 1702, 1608, 1573, 1492, 1450, 1278 cm^{-1}; ^1H NMR ($CDCl_3$ + D_2O, 300 MHz) δ: 7.31–7.36 (t, 4H, Ar); 7.52–7.54 (d, 2H, Ar); 7.71–7.75 (d, 6H, Ar); 8.20–8.30 (t, 4H, Ar). EI MS: No M^+ ion peak. 222 ( ; 2.2%); 221 ( ; 14%); 220 ( ; 100%); 220 ( ; 100%); 195 ( ; 0.8%); 194 ( ; 4.1%); 194 ( ; 4.1%); 193 ( ; 1.6%); 92 ( ; 4.2%). Anal. Calcd for $C_{27}H_{17}N_3O_2$: C, 78.07; H, 4.09; N, 10.12. Found: C, 77.82; H, 4.01; N, 10.31.

4.1.6. Synthesis of 4-(benzo[*d*]oxazol-2-yl)-*N*-(4-methoxy acridin-9-yl)benzamide (3f). Solvent of elution, pet. ether/EtoAc (4:1); white solid (0.300 g, 60%); mp 170 °C; IR (KBr) ν_{max}: 3466, 1610, 1548, 1487, 1445, 1279 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.97 (s, 3H, OCH_3); 7.39–7.46 (m, 4H, Ar); 7.63–7.66 (q, 2H, Ar); 7.79–7.86 (m, 5H, 4HAr + 1H, NH, exch.); 8.16–8.20 (d, 1H, Ar); 8.20–8.43 (m, 4H, Ar). EI MS: No M^+ ion peak: 251 ( ; 0.5%); 222 ( ;15.3%); 221( ; *m*/*z* 251-HCHO; 15%); 220 ( ; 100%); 220 ( ; 100%); 219 ( ; 0.5%); 218 ( ; 0.1%); 209 ( ; 0.4%); 208 ( ; 0.3%); 195 ( ; 1.4%); 194 ( ; 5.8%); 92 ( ; 6.4%). Anal. Calcd for $C_{28}H_{19}N_3O_3$: C, 75.50; H, 4.26; N, 9.43. Found: C, 75.83; H, 4.39; N, 9.71.

4.2. General procedure for 7

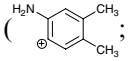
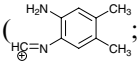
4.2.1. Synthesis of 1-[(2-aminophenylimino) methyl] naphthalen-2-ol (7a). 2-Hydroxynaphthaldehyde (**4**) (172 mg; 1 mmol) and *o*-phenylenediamine (**5a**) (108 mg; 1 mmol) were taken in methanol (5 mL). The reaction contents were irradiated in microwave oven for 30 min at 100-watt power and temperature of the reaction contents was about 63 °C. Crude product so obtained was crystallized from THF to give pure product 1-[(2-aminophenylimino) methyl] naphthalen-2-ol (**7a**).

Similarly were prepared other derivatives, that is, **7b**–**d** and **7e(f)**.

Solvent of crystallization, THF; orange solid (0.300 g, 65%); mp 175 °C; IR (KBr) υ_{max}: 3469, 3374, 1609, 1557, 1475, 1400, 1315 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.70 (br s, 2H, $-NH_2$; exch.); 6.72–6.85 (m, 2H, Ar); 7.06–7.17 (m, 2H, Ar); 7.28–7.36 (m, 2H, Ar); 7.47–7.54 (m, 1H, Ar); 7.72–7.82 (m, 2H, Ar); 8.12–8.14 (d, 1H, Ar); 9.40 (s, 1H, –HC=N). 15.05 (br s, 1H, OH exch.). FAB MS: 263.60 (MH^+; 27); 262.60 (M^+; 22); 119 ( ; 21%); 92 ( ; 15%); 91 ( ; 22%). Anal. Calcd for $C_{17}H_{14}N_2O$: C, 77.86; H, 5.34; N, 10.68. Found: C, 77.62; H, 5.10; N, 10.55.

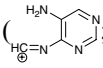
4.2.2. Synthesis of 4-amino-3-[(2-hydroxynaphthalen-1-yl)methylene amino)] benzoic acid (7b). Solvent of crystallization, THF; pale yellow solid (0.300 g, 60%); mp >260 °C; IR (KBr) υ_{max}: 3435, 3334, 1677, 1618, 1400, 1290 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.89 (s, 2H, $-NH_2$ exch.); 6.81–6.84 (d, 1H, Ar); 7.15–7.18 (d, 1H, Ar); 7.37–7.42 (t, 1H, Ar); 7.46–7.66 (m, 2H, Ar); 7.82–7.87 (t, 2H, Ar); 7.97–8.00 (d, 1H, Ar); 8.55–8.58 (d, 1H, Ar); 9.65 (s, 1H, >CH=N–); 15.15 (s, 1H, –OH, exch.). FAB MS 307.67 (MH^+; 65%); 306.70 (M^+; 15%); 305.66 (M^+-H; 8%). Anal. Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.57; N, 9.15. Found: C, 70.71; H, 4.42; N, 9.29.

4.2.3. Synthesis of 1-[(2-amino-5-nitro-phenyl imino) methyl] naphthalen-2-ol (7c). Solvent of crystallization, THF; yellow solid (0.300 g, 75%); mp 225 °C; IR (KBr) υ_{max}: 3469, 3343, 1631, 1484, 1400, 1312 cm^{-1}; ^1H NMR (DMSO-d_6 + D_2O, 300 MHz) δ: 6.86 (d, 1H, Ar); 7.20 (d, 1H, Ar); 7.42 (t, 1H, Ar); 7.63 (m, 1H, Ar); 7.81 (d, 1H, Ar); 7.91 (d, 1H, Ar); 8.01 (dd, 1H, Ar); 8.06 (d, 1H, Ar); 8.30 (d, 1H, Ar); 9.52 (s, 1H, Ar). FAB MS 308.76 (MH^+, 7%). Anal. Calcd for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.23; N, 13.68. Found: C, 66.13; H, 4.01; N, 13.80.

4.2.4. Synthesis of 1-[(2-amino-4,5-dimethylphenylimino) methyl] naphthalen-2-ol (7d). Solvent of crystallization, THF; red solid (0.300 g, 80%); mp 210 °C; IR (KBr) υ_{max}: 3444, 3221, 2917, 1615, 1539, 1486, 1400, 1310 cm^{-1}; ^1H NMR (DMSO-d_6 + $CDCl_3$, 300 MHz) δ: 2.21 (s, 6H, CH_3 + CH_3); 3.40 (br s, 2H, NH_2) 6.64 (s, 1H, Ar); 6.96 (s, 1H, Ar); 7.13–7.14 (d, 1H, Ar); 7.32–7.37 (t, 1H, Ar); 7.50–7.56 (m, 1H, Ar); 7.71–7.82 (dd, 2H, Ar); 8.16–8.18 (d, 1H, Ar); 9.39 (s, 1H, –CH=N–). 15.30 (br s, 1H, –OH, exch.). FAB MS 291.68 (MH^+; 23%); 290.67 (M^+; 28%); 147 ( ; 16%); 120 ( ; 16%). Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.62; H, 6.20; N, 9.65. Found: C, 78.21; H, 6.30; N, 9.90.

4.2.5. Synthesis of 1-[(2-amino-4(5)-chlorophenylimino) methyl] naphthalen-2-ol (7e(f)). Solvent of crystallization, THF; red solid (0.300 g, 60%); mp 230 °C; IR (KBr) υ_{max}: 3447, 1619, 1478, 1400, 1317 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.26 and 5.43 (2s, 2H, $-NH_2$ exch.); 6.68–7.15 (m, 3H, Ar); 7.38–7.59 (m, 3H, Ar); 7.84–7.86 (d, 1H, Ar); 7.96–7.99 (d, 1H, Ar); 8.51–8.60 (q, 1H, Ar); 9.61–9.65 (d,1H, –HC=N–); 15.29–15.35 (d, 1H, OH exch.). Two singlets at 5.26 and 5.43 show existence the of two diastereomers. Also a doublet for –CH=N– at δ 9.61–9.65 indicates the presence of diastereomers. FAB MS 297.92 (MH^+; 32%). Anal. Calcd for $C_{17}H_{13}N_2OCl$: C, 68.80; H, 4.38; N, 9.44. Found: C, 69.02; H, 4.60; N, 9.21.

4.2.6. Synthesis of 1-[(4-aminopyrimidin-5-yl-imino) methyl] naphthalen-2-ol (9). 2-Hydroxynaphthaldehyde (**4**) (172 mg; 1 mmol) and 4,5-diaminopyrimidine (**8**) (110 mg; 1 mmol) were taken in methanol (5 mL). The reaction contents were irradiated in microwave oven for 30 min at 100-watt power and temperature of the reaction contents was about 63 °C. Crude product so obtained was crystallized from THF to give pure product 1-[(4-aminopyrimidin-5-yl-imino) methyl] naphthalen-2-ol (**9**).

Solvent of crystallization, THF; red solid (0.300 g, 86%); mp 240 °C; IR (KBr) υ_{max}: 3460, 3321, 1653, 1580, 1508, 1398 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 6.98–8.32 (m, 7H, Ar); 8.54–8.57 (d, 1H, Ar); 9.66 (s, 1H, –CH=N–); 14.29 (s, 1H, OH, exch.); NH_2 is expected under water peak. FAB MS 265.59 (MH^+; 32%); 121 ( ; 33%). Anal. Calcd for $C_{15}H_{12}N_4O$: C, 68.18; H, 4.54; N, 21.21. Found: C, 68.00; H, 4.61; N, 21.02.

4.2.7. Synthesis of 4,4′bis [1-{(2-aminophenylimino) methyl] naphthalen-2-ol (11). 2-Hydroxynaphthaldehyde (**4**) (172 mg; 1 mmol) and 3,3′-diaminobenzidine (**8**) (214 mg; 1 mmol) were taken in methanol (5 mL). The reaction contents were irradiated in microwave oven for 30 min at 100-watt power and temperature of the reaction content was about 63 °C. Crude product so obtained was crystallized from THF to give pure product 4,4′bis[1-{(2-aminophenylimino) methyl] naphthalen-2-ol (**11**).

Solvent of crystallization, THF; red solid (0.300 g, 85%); mp 235 °C; IR (KBr) υ_{max}: 3439, 3197, 1607, 1485, 1400, 1312 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.26 (br s, 4H, NH_2 + NH_2 exch.); 6.94–7.19 (q, 4H, Ar); 7.34–7.89 (m, 12H, Ar); 8.39 (d, 2H, Ar); 9.53–9.61 (d, 2H, 2×–CH=N–). FAB MS 523.73 (MH^+; 2%); 522.73

(M^+; 3%). Anal. Calcd for $C_{34}H_{26}N_4O_2$: C, 78.16; H, 4.98; N, 10.72. Found: C, 78.41; H, 5.03; N, 10.51.

4.3. Anti-inflammatory activity evaluation

Anti-inflammatory activity evaluation[27] 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 plantar apponeurosis. The volume of the paw was measured plethysmographically immediately after and three hours 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 that of the group receiving a standard drug.

Compounds **3a–f**, **7a–e(f)** and **11** at 50 mg/kg po suspended in 2% gum acacia exhibited 0.0%, 3.5%, 7.5%, 0.0%, 0.0%, 0.0%, 26.6%, 25.5%, 28.2%, 28.2%, 31.4% and 35.8%, respectively, anti-inflammatory activity (Table 1) compared to ibuprofen which showed 38.8% activity at 50 mg/kg po.

4.4. Analgesic activity evaluation

Analgesia was measured by the writhing assay[28] using Swiss mice (15–20 g). Female mice were screened for writhing on day 1 by injecting intraperitoneally 0.2 cm^3 of 0.02% aqueous solution of phenylquinone. They were kept on flat surface and the number of writhes of each mouse was recorded for 20 min. The mice showing significant writhes (>10) 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 po dose suspended in 2% gum acacia 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 the following formula.

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

This was taken as a percent of analgesic response and was averaged in each group of mice. Percent of animals exhibiting analgesia was determined with each dose.

Compounds **3a–f**, **7a–e(f)** and **11** were screened for analgesic activity at 50 mg/kg po. All these compounds exhibited 25%, 25%, 0.0%, 25%, 25%, 25%, 30%, 20%, 40%, 30%, 60% and 50% analgesic activity, respectively (Table 1), as compared to ibuprofen which exhibited 50% activity.

4.5. Kinase inhibition activity evaluation[29–31]

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

4.5.2. Buffers

4.5.2.1. Homogenization buffer. 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate 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.

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

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

4.5.3. 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 10 min incubation. The activities were calculated in % of the maximal activity, that is, in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethylsulfoxide.

4.5.3.1. GSK-3α/β. GSK-3α/β was either purified from porcine brain by affinity purification on an immobilized fragment of axin.[29] It was assayed, following a 1/100 dilution in 1 mg BSA/mL, 10 mM DTT, with 5 μL of 40μM 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.

4.5.3.2. CDK-1/cyclin B. CDK-1/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.[30,31] The kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in the presence of 15 μM [γ-^{32}P]ATP (3000 Ci/mmol; 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.

4.5.3.3. CDK-5/p25. CDK-5/p25 was reconstituted by mixing equal amounts of recombinant mammalian CDK-5 and p25 expressed in *Escherichia coli* as GST

(glutathione-*S*-transferase) fusion proteins and purified by affinity chromatography on glutathione–agarose (vectors kindly provided by Dr. J. H. Wang) (p25 is a truncated version of p35, the 35KDa CDK-5 activator). Its activity was assayed in buffer C as described for CDK-1/cyclin B.

Compounds **3a**–**f**, **7a**–**e**(**f**) and **11** were screened for CDK-1, CDK-5 and GSK-3 inhibition activity and IC_{50} values are reported in Table 1. Compounds **3a** for CDK-5 (IC_{50} 7.5 μM) and **3b** for CDK-1 (IC_{50} 7.4 μM) and CDK-5 (IC_{50} 4.6 μM) exhibited some interesting inhibition activities.

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