

Synthesis and potent antimicrobial activity of some novel 2-phenyl or methyl-4H-1-benzopyran-4-ones carrying amidinobenzimidazoles

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Abstract—Series of flavones and methyl-4H-1-benzopyran-4-ones carrying mono or diamidinobenzimidazoles at different positions were synthesized and evaluated for antibacterial and antifungal activities against *E. coli*, *S. aureus*, MRSA (methicillin-resistant *S. aureus*), MRSE (methicillin-resistant *S. epidermidis*), *S. faecalis* and *C. albicans*, *C. krusei*. The results showed that while all diamidines are inactive, the compounds having monoamidinobenzimidazoles at the C-6 position of the 2-phenyl-4H-1-benzopyran-4-one have potent antibacterial activities, particularly, against Gram-positive bacteria. Compounds **23** and **22** exhibited the best inhibitory activity with MIC values of 1.56 µg/mL against *S. aureus*, MRSA, MRSE and 3.12 µg/mL against *C. albicans*, respectively.
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1. Introduction

In our efforts to discover novel antimicrobial agents, we have screened series of mono and diamidinobenzimidazoles linked to various flavone nuclei. Flavonoids are a group of benzo- γ -pyrone derivatives which exhibit a wide spectrum of biological activity. Several recent papers report the presence of antibacterial activity for this class of compounds.¹ Thus, the retrochalcone licochalcone is active against *Staphylococcus aureus* with a MIC of 6.25 µg/mL. Also, 5,7-dihydroxy-3,8-dimethoxyflavone has an MIC of 50 µg/mL against *S. epidermidis*. Another of the flavonone² derivatives inhibits the growth of *S. aureus* with MIC of 1.56–6.25 µg/mL, and is effective against MRSA (methicillin-resistant strains of *S. aureus*) as well. A series of 38 plant-derived flavonoids representing seven different structural groups were tested against antibiotic-resistant bacteria MRSA, VRE (vancomycin-resistant enterococci), multidrug-resistant *Burkholderia cepacia* and *Klebsiella pneumoniae*.³ Among them myricetin displayed the best inhibitory activity with MIC values ranging from 32 to

256 µg/mL. In addition several flavonolignans and simple alkylated flavones were prepared and were shown to be potent inhibitors of the NorA MDR(multi drug resistance) efflux pump in *S. aureus*.⁴ In other study, a series of flavones was synthesized for their DNA-gyrase inhibitory and antibacterial activities.⁵ This led to the identification of compounds with potent *Escherichia coli* DNA-gyrase inhibitory activity, and significant antimicrobial activity. The most active compound, ellagic acid has an IC₅₀ = 3.3 µg/mL, which is comparable to some of the currently marketed 4-quinolone antibacterials. Later, a series of novel aza-analogues of flavones were reported as topoisomerase inhibitors from the same laboratory.⁶ Recently, possible modes of antimicrobial action mechanism of flavonoids have been discussed in a review.⁷ The antimicrobial properties of dicationic arylamidines against a number of pathogens including protozoa, bacteria and fungi have been reported.⁸ Among the dicationic molecules pentamidine, has seen significant clinical use. Pentamidine has been used clinically against African trypanosomiasis, antimony-resistant leishmaniasis and *P. carinii* pneumonia.⁸ This class of dicationic molecules has been shown to bind to the minor-groove of DNA at AT-rich sites. This binding has been postulated to be important in the mode of antimicrobial action of these compounds possibly leading to inhibition of DNA dependent enzymes

Keywords: Methicillin-resistant *S. aureus*; Methicillin-resistant *S. epidermidis*; 4H-1-Benzopyran-4-one; Amidinobenzimidazoles.

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(i.e., topoisomerases, nucleases) or possibly by direct inhibition of transcription.^{8,9} In our previous work many dicationic amidines have been found to be highly effective against *P. carinii*, *Cryptosporidium parvum*, *C. albicans*, *Cryptococcus neoformans*,^{8,9} *Trypanosoma b. rhodesiense*,¹⁰ *Trypanosoma cruzi*¹¹ and *Leishmania donovani*.¹¹ On the other hand, mono amidinobenzimidazoles have been identified as inhibitors of the bacterial KinA/SpoOF two-component system TCS (which include a histidine protein kinase HPK and a response regulator RR) which are responsible for adapting bacteria to the environmental changes and survival within the host.¹² Many of these inhibitors display good in vitro antibacterial activity in particular, against Gram-positive bacteria (i.e., *S. aureus*, MRSA, VRE).

Based on the above mentioned studies, we have synthesized a series of compounds carrying either mono- or di-N-alkyl substituted amidinobenzimidazoles linked to the 4H-1-benzopyran-4-one nucleus in order to investigate their antimicrobial activity.

2. Chemistry

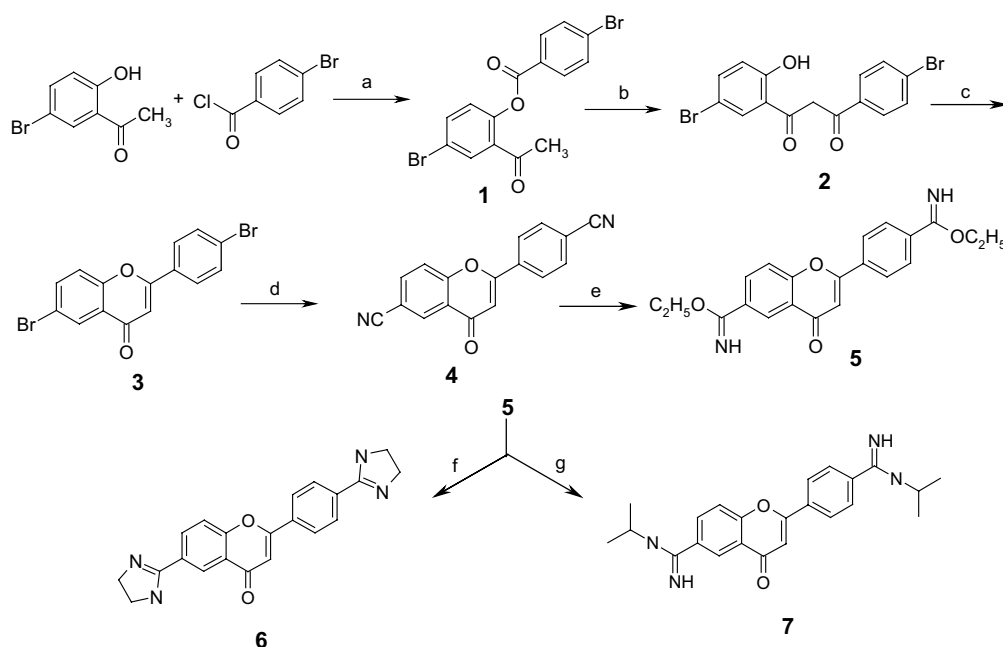
For the synthesis of 4H-1-benzopyran-4-one (**3**), the Baker–Venkataraman¹³ rearrangement, a general procedure for the preparation of flavones, was chosen (Scheme 1). 5'-Bromo-2'-hydroxy-acetophenone was first converted into a 4-bromobenzoyl ester (**1**) and this intermediate was then treated with KOH/pyridine, which formed 1,3-diketone (**2**). Treatment of the diketone with concd H₂SO₄ lead to formation of the dibrominated flavone (**3**).¹⁴ The reaction of the dibromide with copper(I) cyanide gave the corresponding bis nitrile (**4**). The bis nitrile was converted into the imidate ester **5**,

using a modified Pinner method, and the imidate ester was used directly without characterization to make the amidines. The dicationic amidine derivatives **6** and **7** were obtained by reacting the imidate esters with appropriate amines (Scheme 1). 2-(4-Methylphenyl)-7-methoxy-4H-1-benzopyran-4-one **10**, was synthesized from **8** and **9**, by a similar procedure and is outlined in Scheme 2. Compound **10** was converted into 4'-bromomethylflavone **11**, by reaction with *N*-bromosuccinimide in the presence of benzoyl peroxide. Treatment of this compound with hexamethylenetetramine in glacial acetic acid gave 2-(4-formylphenyl)-7-methoxy-4H-1-benzopyran-4-one **12**. The known 4H-1-benzopyran-4-one aldehydes **13**,¹⁵ **17**,¹⁶ **19**¹⁵ and **26**¹⁵ were reacted with *N*-alkyl substituted-3,4-diaminobenzamidines in the presence of 1,4-benzoquinone to give the targeted benzimidazoles (Scheme 3).

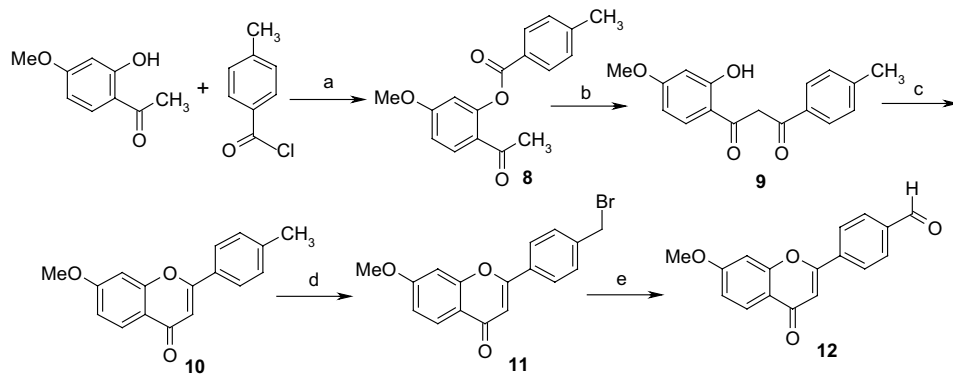
2,6-Dimethylchromone **30**,¹⁷ was prepared from 2-acetoacetyl-4-methylphenol **29**,¹³ as described in the literature (Scheme 4). Mono bromination of this compound with one equivalent of *N*-bromosuccinimide gave the **31**. The reaction of this compound with hexamethylenetetramine afforded **32** as previously described for **12**. The aldehyde was reacted with 3,4-diamino-*N*-isopropyl or butylbenzamidine in the presence of 1,4-benzoquinone to give the benzimidazoles **33** and **34**, respectively.

3. Microbiology

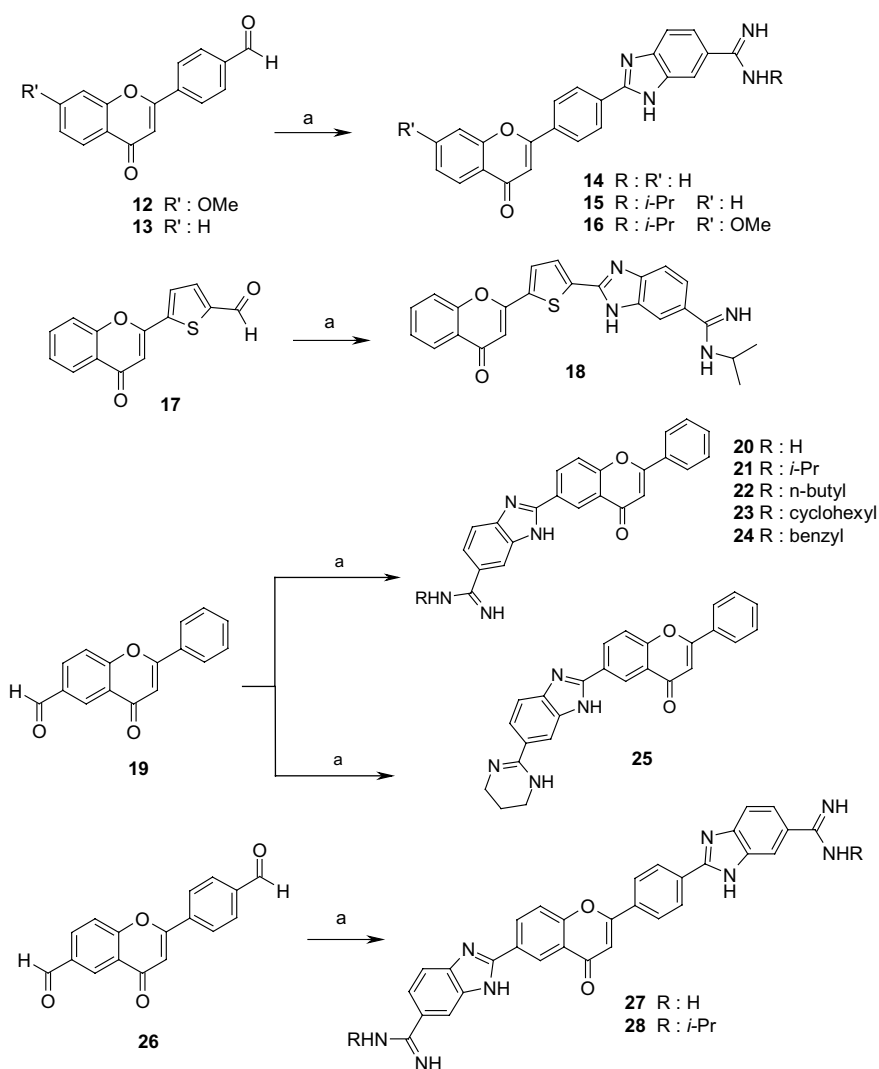
The benzimidazoles **6–34** were tested in vitro for antibacterial activity against Gram-positive *S. aureus*, methicillin-resistant *S. aureus* (MRSA, clinical isolate), methicillin-resistant *S. epidermidis* (MRSE, clinical isolate), *S. faecalis*, Gram-negative *E. coli* bacteria and



Scheme 1. Reagents: (a) pyridine; (b) powder KOH/pyridine; (c) concd sulfuric acid; (d) CuCN; (e) HCl gas; (f) ethylenediamine; (g) isopropylamine.



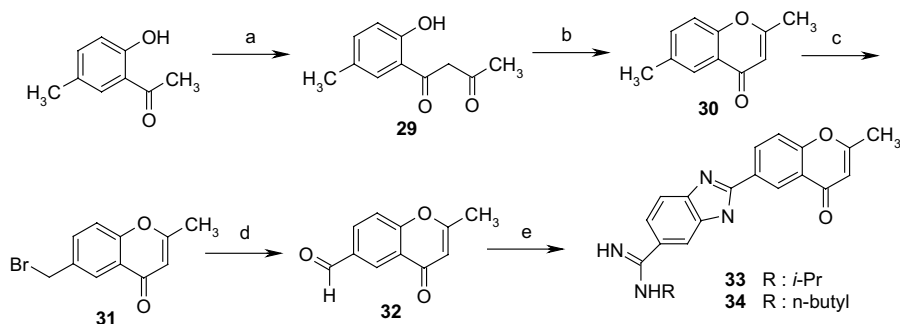
Scheme 2. Reagents: (a) pyridine; (b) powder KOH/pyridine; (c) concd sulfuric acid; (d) *N*-bromosuccinimide; (e) hexamethylenetetramine.



Scheme 3. Reagents: (a) 1,4-benzoquinone/corresponding 3,4-diaminobenzamidine derivatives.

for antifungal activity against *Candida albicans* by the macro-broth dilution^{18,19} assay to determine the MICs (listed in Table 1). The synthesized compounds and reference drugs were dissolved in DMSO–water (50%), at a concentration of 400 g/mL. The concentration was adjusted to 100 µg/mL by 4-fold dilution with media

culture and bacteria solution. Data were not taken for the initial solution because of the high DMSO concentration (12.5%). As shown in Table 1, none of the compounds have inhibitory effect against *E. coli*. However, compounds carrying amidinobenzimidazoles at position C-6 of 2-phenyl-4H-1-benzopyran-4-ones



Scheme 4. Reagents: (a) sodium hydride/EtOAc; (b) $\text{CH}_3\text{COOH}/\text{HCl}$; (c) *N*-bromosuccinimide; (d) hexamethylenetetramine; (e) 1,4-benzoquinone/3,4-diamino-*N*-isopropylbenzamidines or 3,4-diamino-*N*-butyllbenzamidines.

Table 1. Antibacterial and antifungal activity of compounds **6–34** (MIC, minimum inhibitory concentration $\mu\text{g/mL}$)

Compound	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 29213	MRSA*	MRSE**	<i>S. faecalis</i> ATCC 29212	<i>C. albicans</i> ATCC 10231	<i>C. krusei</i> ATCC 10231
6	>50	>50	>50	NT	NT	>50	>50
7	50	>50	50	NT	NT	>50	>50
14	>50	50	50	NT	NT	25	>50
15	>50	12.5	12.5	25	50	6.25	12.5
16	>50	50	50	25	50	12.5	>50
18	>50	25	25	12.5	50	12.5	>50
20	50	12.5	25	12.5	25	12.5	50
21	>50	6.25	12.5	3.12	25	12.5	25
22	25	6.25	3.12	3.12	12.5	3.12	6.25
23	>50	1.56	1.56	1.56	12.5	12.5	25
24	25	6.25	3.12	1.56	6.25	6.25	25
25	>50	25	25	3.12	25	6.25	12.5
27	>50	50	50	NT	NT	>50	>50
28	>50	50	50	NT	NT	>50	50
33	>50	>50	>50	50	50	25	50
34	>50	50	50	50	50	25	50
Ampicillin		0.39	50		0.78		
Fluconazol						3.12	50
Gentamisin	0.78						
Sultamisilin		0.78	25	3.12	1.56		

MRSA*: methicillin-resistant *S. aureus* (clinical isolate).

MRSE*: methicillin-resistant *S. epidermidis* (clinical isolate).

NT: not tested.

20–24, showed significant activity, in particular against *S. aureus*, methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE). Among them compound **23** is the most active with a MIC value of 1.56 $\mu\text{g/mL}$ against *S. aureus* and both of the drug-resistant bacteria. While ampicillin and sultamisilin is practically inactive against MRSA (50 and 25 $\mu\text{g/mL}$), all the compounds belonging to the series **20–24** are effective towards MRSA. Less activity was noted against *S. faecalis*, the most potent compound was **24** with 6.25 $\mu\text{g/mL}$ MIC value. According to the obtained results, bulky alkylated amidines such as cyclohexyl or *n*-butyl results yield increased activity. Interestingly, compounds in the isomeric series, where the benzimidazole system is linked to the 2-phenyl ring rather than the benzopyran-4-one (**14–16**, **18**), were not active. In contrast to the broad spectrum antimicrobial activity noted for other diamidines, compounds **6**, **7**, **27** and **28** show no significant activity against bacteria and fungi. Meanwhile, compound **22** gave the best inhibitory activity with MIC values of 3.12 and 6.25 $\mu\text{g/mL}$ against *C.*

albicans and *C. krusei*, respectively. Generally, all of the compounds showed somewhat less antifungal than antibacterial activity. Compounds **33** and **34**, which have a 2-methyl instead of 2-phenyl on the benzopyran-4-one moiety, show much less activity than the corresponding compounds **21** and **22**. This result demonstrates the importance of the 2-phenyl group in this series. Furthermore, in one of our recently published article,²⁰ we have reported that this class of the cationic 4H-1-benzopyran-4-one-substituted benzimidazoles exhibited no cytotoxic effects.

4. Conclusion

This work demonstrates that monoamidines in the benzimidazole flavone series show a good activity profile versus Gram-positive bacteria. Compounds **22** and **23**, having *N*-bulky alkyl substituted amidinobenzimidazoles at the position C-6, of 2-phenyl-4H-1-benzopyran-4-one exhibited the greatest activity against *S. aureus*,

methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) with MIC values of 1.56 µg/mL. In vivo and mode of action mechanism studies for compounds **22** and **23** are necessary to fully understand and to expand upon their potent antibacterial activities.

5. Experimental

Uncorrected melting points were measured on a Mel Temp 3.0 capillary melting point apparatus. ¹H NMR and ¹³C NMR spectra were recorded employing Varian GX400 spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (*J*) are reported in hertz. FAB, MS(CI) and ESI mass spectra were recorded at Georgia Institute of Technology, Atlanta, GA and Ankara University Faculty of Pharmacy (Waters Micromass ZQ), respectively. Microanalyses were performed by Atlantic Microlab Inc. (Norcross, GA) and were within ±0.4% of calculated values. All chemicals and solvents were purchased from Aldrich Chemical Co., or Fischer Scientific.

5.1. 4-Bromobenzoic acid 2'-acetyl-4'-bromophenyl ester (**1**)

p-Bromobenzoyl chloride (5 g, 22.7 mmol) was added to a mixture of 5-bromo-2-hydroxy-acetophenone (4.89 g, 22.7 mmol) in pyridine (10 mL) and heated for 0.5 h, at 80 °C. The mixture was cooled and poured into the water, washed with dilute Na₂CO₃ solution and water, recrystallization from EtOH to give **1** (8.2 g, 88.7%) as a white needle: mp 139–140 °C; Anal. (C₁₅H₁₀Br₂O₃) C, H.

5.2. 1-(5-Bromo-2-hydroxyphenyl)-3-(4-bromophenyl)-1,3-propanedione (**2**)

Compound **1** (8 g, 20 mmol) in 60 mL pyridine and powdered KOH (2 g) were stirred for 1 h at 60 °C, the reaction mixture was cooled, water was added and the solution pH adjusted to 5 with HCl acid. The yellow precipitate was filtered and washed with water. Recrystallization from acetone–MeOH to give **2** (5.94 g, 74.3%); mp 155–157 °C; Anal. (C₁₅H₁₀Br₂O₃) C, H.

5.3. 6-Bromo-2-(4-bromophenyl)-4H-1-benzopyran-4-one (**3**)

A mixture of **2** (5.5 g, 13.8 mmol) and concd H₂SO₄ (15 mL) was stirred at room temperature for 20 min. Water was added and precipitate was collected, recrystallization from EtOH to give **3** (4.69 g, 89.5%) as white needles: mp 253 °C; ¹H NMR (DMSO-*d*₆) δ 7.01 (s, 1H), 7.79 (3H), 7.99 (dd, 1H, *J*_o = 8.8, *J*_m = 2.3), 8.05 (d, 2H), 8.11 (d, 1H, *J* = 2.3); Anal. (C₁₅H₈Br₂O₂) C, H.

5.4. 6-Cyano-2-(4-cyanophenyl)-4H-1-benzopyran-4-one (**4**)

A mixture of **3** (4.5 g, 11.8 mmol) and copper(I) cyanide (2.2 g, 24.71 mmol) in 1-methyl-2-pyrrolidinone (25 mL)

was heated 3 h under nitrogen. The reaction mixture was poured into ice water. The green coloured precipitate was filtered off and washed with water. The solid was stirred in water containing ethylenediamine (15 mL) for 1 h, filtered and washed with water, then the solid was stirred in a solution of KCN (7.5 g) in water (100 mL) for overnight. The dinitrile was filtered, washed with water (2.98 g), dried, dissolved in (600 mL) the mixture of CHCl₃–EtOAc (30:1) and chromatographed over neutral Al₂O₃ to yield a white fluffy solid 2.27 g (70.7%); mp 339–340 °C; ¹H NMR (DMSO-*d*₆) δ 7.28 (s, 1H), 8.0 (d, 1H, *J* = 8.73), 8.05 (d, 2H, *J* = 8.25), 8.24 (dd, 1H, *J*_o = 8.7, *J*_m = 2.1), 8.3 (d, 2H, *J* = 8.3), 8.43 (d, 1H, *J* = 2.1); Anal. (C₁₇H₈N₂O₂·0.25 H₂O) C, H, N.

5.5. 6-(2-Imidazolyl)-2-4-(2-imidazolyl)phenyl-4H-1-benzopyran-4-one hydrochloride (**6**)

The bis nitrile **4** (0.78 g, 2.87 mmol) was suspended in a mixture of absolute EtOH and CH₂Cl₂ (30 mL, 50%), cooled in ice bath and dry HCl gas was passed through it. When the suspension was saturated with HCl(g), the flask was stoppered and the contents were stirred at room temperature until the TLC monitored the disappearance of the bis nitrile (7 days). Ether was added, then the suspension was collected by filtration, washed with anhydrous Et₂O and dried in vacuo to give bis imide ester HCl, **5**. A mixture of ethylenediamine (0.23 g, 3.8 mmol) and **5** (0.64 g, 1.68 mmol) in 15 mL of EtOH was heated at reflux for 12 h. A white solid separated which was filtered and washed with EtOH. The solid was treated with water and the mixture was basified with dilute NaOH solution, the solid filtered, washed with water and dried, mp >350 °C. Free base was suspended in absolute EtOH saturated with HCl gas and the mixture was heated for 10 min. After cooling ether was added and the solid was collected by filtration, washed with ether and dried in vacuo, yield 0.2 g (26.7%) of white powder: mp >370 °C; ¹H NMR (DMSO-*d*₆) δ 4.19 (8H), 7.25 (s, 1H), 8.09 (d, 1H), 8.11 (d, 2H, *J* = 8.8), 8.33 (dd, 1H, *J*_o = 8.7, *J*_m = 2.1), 8.37 (d, 2H, *J* = 8.6), 8.66 (d, 1H, *J* = 2.2); ¹³C NMR (DMSO-*d*₆): 178.3, 165.5, 165.1, 163.01, 159.6, 136.7, 134.6, 130.09, 128.3, 127.5, 125.9, 124.2, 121.4, 120.5, 109.9, 45.6; MS (FAB) *m/z* 358.8 (M⁺); Anal. (C₂₁H₁₈N₄O₂·2HCl·HOH) C, H, N.

5.6. 6-Isopropylamidino-2-(4-isopropylamidinophenyl)-4H-1-benzopyran-4-one hydrochloride (**7**)

A mixture of freshly distilled isopropylamine (0.355 g, 6 mmol) and **5** (0.64 g, 1.68 mmol) in 15 mL of EtOH was heated at reflux for 12 h. A white solid separated and was filtered and washed with EtOH. The solid was treated with water and the mixture was basified with dilute NaOH solution, the solid filtered, washed with water and dried, mp 358 °C. Free base was suspended in absolute EtOH saturated with HCl and the mixture was heated for 5 min. After cooling dry ether was added and the solid collected by filtration, washed with ether and dried in vacuo, yield 0.643 g (46.03%) of white powder: mp 381–382 °C; ¹H NMR (DMSO-*d*₆) δ 1.31 and

1.33 (12H), 4.16 (m, 2H, $J = 6.6$), 7.37 (s, 1H), 7.99 (d, 2H, $J = 8.42$), 8.08 (d, 1H, $J = 8.5$), 8.22 (dd, 1H, $J_o = 8.2$, $J_m = 2.7$), 8.37 (d, 2H, $J = 8.24$), 8.44 (d, 1H, $J = 1.95$), 9.37 (d, 1H), 9.72 (s, 1H), 9.89 (d, 1H); ^{13}C NMR (DMSO- d_6) δ 179.3, 164.1, 163.1, 162.7, 159.7, 136.4, 135.5, 133.6, 130.6, 128.8, 128.07, 127.5, 124.59, 121.69, 110.19, 47.2, 22.6; MS (FAB): m/z 390.9 (M^+); Anal. ($\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_2 \cdot 2\text{HCl} \cdot 0.5\text{C}_2\text{H}_5\text{OH}$) C, H, N.

5.7. 4-Methylbenzoic acid 2'-acetyl-5'-methoxyphenyl ester (8)

A mixture of 2-hydroxy-4-methoxyacetophenone (5 g, 30 mmol) and *p*-toluoyl chloride (4.65 g, 30 mmol) in pyridine (10 mL) were heated for 0.5 h at 80 °C. The mixture was cooled and poured into ice water, acidified with HCl acid, precipitate was filtered washed with water, recrystallization from EtOH gave **8** (7.8 g, 91.5%), as white crystals: mp 66–67 °C; Anal. ($\text{C}_{17}\text{H}_{16}\text{O}_4$) C, H.

5.8. 1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methylphenyl)-1,3-propanedione (9)

Compound **8** (7.1 g, 25 mmol) in 40 mL pyridine and powdered KOH (3 g) were stirred for 1 h at 60 °C, the reaction mixture was cooled, water was added and the solution pH adjusted to 5 with HCl. The yellow precipitate was filtered and washed with water. Recrystallization from EtOH gave **9** (4.5 g, 63.4%), as yellow crystals: mp 123–125 °C; Anal. ($\text{C}_{17}\text{H}_{16}\text{O}_4$) C, H.

5.9. 7-Methoxy-2-(4-methylphenyl)-4H-1-benzopyran-4-one (10)

Compound **9** (4 g, 14 mmol) in sulfuric acid (98%, 20 mL) were stirred for 1 h at room temperature. Water was added, the precipitate was collected and recrystallization from EtOH gave **10** (3.37 g, 90%) as white powder: mp 133–134 °C; Anal. ($\text{C}_{17}\text{H}_{14}\text{O}_3$) C, H.

5.10. 2-(4-Bromomethylphenyl)-7-methoxy-4H-1-benzopyran-4-one (11)

The mixture of **10** (2 g, 7.52 mmol), *N*-bromosuccinimide (1.34 g, 7.52 mmol) and catalytic amount of benzoyl peroxide in CCl_4 (30 mL) was heated at reflux for 6 h. The mixture was filtered while it was hot and CCl_4 was evaporated. Recrystallization of the solid residue from EtOAc-*n*-hexane (50%) two times, gave pure compound as white needles, yield (0.86 g, 33.2%): mp 187–188 °C; MS (ESI) m/z 345 and 347 ($\text{M}+1$, 31), 289 (100), MS (APCI) m/z 345 and 347 ($\text{M}+1$, 48), 141 (100); Anal. ($\text{C}_{17}\text{H}_{13}\text{BrO}_3$) C, H.

5.11. 2-(4-Formylphenyl)-7-methoxy-4H-1-benzopyran-4-one (12)

A mixture of **11** (0.85 g, 2.46 mmol) and hexamethylenetetramine (4 g, 28.6 mmol) in 30 mL of acetic acid (50%) was heated at reflux for 4 h. HCl (10 mL, 50%) was added and refluxing was continued for 0.5 h. The reaction mixture was diluted with water and precipitate

was collected. Recrystallization of the solid from EtOAc-*n*-hexane (50%) gave **12** as white powder, yield (0.5 g, 58.4%): mp 183–184 °C; MS (EI) m/z 280 ($\text{M}+100$), 252 (52.4), 237 (56.9), 209 (6.3), 150 (23.6); Anal. ($\text{C}_{17}\text{H}_{12}\text{O}_4$) C, H.

5.12. General method for the synthesis of 14–16, 18, 20–25

A solution of related mono aldehyde derivatives (1.5 mmol), the corresponding *N*-substituted-amidino-1,2-phenylenediamine HCl-0.5HOH (1.5 mmol), and 1,4-benzoquinone (1.5 mmol) in EtOH (20 mL) was heated at reflux for 12 h. The reaction mixture was cooled to room temperature, and the blue coloured solid was collected by filtration, washed with cold EtOH and the solid stirred in dilute sodium hydroxide (1 M, 40 mL) for 5 h. The precipitate was washed with water, until the colour completely disappeared and the solid was dried. Free base was dissolved in hot EtOH (200 mL), boiled with charcoal and filtered. The filtrate volume was reduced to 50 mL and acidified with HCl gas and the white powder solid was collected.

5.12.1. 2-[4-(5(6)-Amidinobenzimidazolyl)phenyl]-4H-1-benzopyran-4-one hydrochloride (14). Yield 0.27 g, 38.2%, white: mp >350 °C; ^1H NMR (DMSO- d_6) δ 7.17 (s, 1H), 7.5 (m, 1H), 7.83 (m, 3H), 7.91 (d, 1H, $J = 8.1$), 8.05 (dd, 1H), 8.3 (d, 1H, $J = 1.4$), 8.35 (d, 2H, $J = 8.5$), 8.6 (d, 2H, $J = 8.7$), 9.29 (s, 2H), 9.52 (s, 2H); ^{13}C NMR δ 177.1, 165.6, 161, 155.6, 151.4, 138.6, 135.5, 134.4, 134.1, 128.5, 128.3, 127.1, 125.6, 124.7, 124, 123.5, 123.3, 118.6, 115.8, 114.7, 107.9; MS (FAB) m/z 380.8 (M^+); Anal. ($\text{C}_{23}\text{H}_{16}\text{N}_4\text{O}_2 \cdot 2\text{HCl} \cdot \text{HOH}$) C, H, N.

5.12.2. 2-[4-(5(6)-Isopropylamidinobenzimidazolyl)-phenyl]-4H-1-benzopyran-4-one hydrochloride (15). Yield 0.267 g, 35.6%, white; mp >350 °C; ^1H NMR (DMSO- d_6) δ 1.31 (d, 6H, $J = 6.4$), 4.12 (m, 1H, $J = 6.3$), 7.19 (s, 1H), 7.5 (m, 1H), 7.67 (dd, 1H), 7.85 (m, 3H), 8.05 (dd, 1H), 8.12 (d, 1H), 8.35 (d, 2H, $J = 8.6$), 8.58 (d, 2H, 8.7), 9.12 (s, 1H), 9.5 (s, 1H), 9.65 (d, 1H); ^{13}C NMR (DMSO- d_6) δ 177.1, 162.1, 161.3, 155.7, 151.7, 139.3, 136.3, 134.4, 133.8, 129.5, 128.2, 127.1, 125.5, 124.8, 124.5, 123.9, 123.4, 118.6, 116.1, 114.7, 107.9, 45.2, 21.3; MS (FAB) m/z 422.8 (M^+); Anal. ($\text{C}_{26}\text{H}_{22}\text{N}_4\text{O}_2 \cdot 2\text{HCl} \cdot 0.25\text{HOH}$) C, H, N.

5.12.3. 2-[4-(5(6)-Isopropylamidinobenzimidazolyl)-phenyl]-7-methoxy-4H-1-benzopyran-4-one hydrochloride (16). Yield 0.28 g, 36.7%, white: mp >330–332 °C; ^1H NMR (DMSO- d_6) δ 1.296 (d, 6H, $J = 6.2$), 3.92 (s, 3H), 4.12 (m, 1H, $J = 6.3$), 7.13 (s, 1H), 7.19 (s, 1H), 7.35 (m, 1H), 7.56 (d, 1H), 7.8 (d, 1H), 7.92 (dd, 1H), 8.14 (s, 1H), 8.32 (d, 2H, $J = 8.6$), 8.45 (d, 2H, 8.7), 8.99 (s, 1H), 9.4 (s, 1H), 9.5 (d, 1H); MS (FAB) m/z 453 ($\text{M}+1$); Anal. ($\text{C}_{27}\text{H}_{24}\text{N}_4\text{O}_3 \cdot \text{HCl} \cdot 0.25\text{EtOH} \cdot 0.5\text{HOH}$) C, H, N.

5.12.4. 2-[5-(5(6)-Isopropylamidinobenzimidazolyl)thiophenyl]-4H-1-benzopyran-4-one hydrochloride (18). Yield 0.31 g, 40.3%, yellow: mp >350 °C; ^1H NMR(DMSO-

d_6) δ 1.27 (d, 6H, $J = 6.53$), 7.01 (s, 1H), 7.47 (td, 1H), 7.53 (dd, 1H), 7.70 (d, 1H), 7.75 (d, 1H), 7.81 (td, 1H), 7.97 (s, 1H), 8.01 (dd, 1H), 8.25 (d, 1H), 8.28 (d, 1H), 8.9 (s, 1H), 9.45 (s, 1H), 9.5 (d, 1H); MS (FAB) m/z 429 (M+1). Anal. ($C_{24}H_{20}N_4O_2S \cdot 2HCl \cdot 0.75HOH$) C, H, N.

5.12.5. 6-[2-(5(6)-Amidinobenzimidazolyl)]-2-phenyl-4H-1-benzopyran-4-one hydrochloride (20). Yield 0.28 g, 40.6%, white: mp $>350^\circ C$; 1H NMR (DMSO- d_6) δ 7.08 (s, 1H), 7.62 (3H), 7.76 (dd, 1H, $J_o = 8.5$, $J_m = 1.9$), 7.83 (d, 1H, $J = 8.6$), 7.97 (d, 1H, $J = 8.73$), 8.12 (2H), 8.24 (d, 1H, $J_m = 1.8$), 8.75 (dd, 1H, $J_o = 8.7$, $J_m = 2.1$), 8.94 (1H), 9.2 (br s, 1H), 9.4 (br s, 1H); MS (FAB) m/z 380.8 (M⁺). Anal. ($C_{23}H_{16}N_4O_2 \cdot 2HCl \cdot 0.3HOH$) C, H, N.

5.12.6. 6-[2-(5(6)-Isopropylamidinobenzimidazolyl)]-2-phenyl-4H-1-benzopyran-4-one hydrochloride (21). Yield 0.16 g, 21.6%, white: mp $>350^\circ C$; 1H NMR (DMSO- d_6) δ 1.342 (d, 6H, $J = 6.4$), 4.2 (m, 1H), 7.09 (s, 1H), 7.61 (3H), 7.68 (dd, 1H, $J_o = 8.5$, $J_m = 1.9$), 7.85 (d, 1H, $J = 8.6$), 8.05 (d, 1H, $J = 8.73$), 8.13 (3H), 8.83 (dd, 1H, $J_o = 8.7$, $J_m = 2.1$), 8.97 (d, 1H, $J = 2$), 9.2 (br s, 1H), 9.52 (br s, 1H), 9.66 (br s, 1H); MS (FAB) m/z 422.8 (M⁺); Anal. ($C_{26}H_{22}N_4O_2 \cdot 2HCl$) C, H, N.

5.12.7. 6-[2-(5(6)-Butylamidinobenzimidazolyl)]-2-phenyl-4H-1-benzopyran-4-one hydrochloride (22). Yield 0.36 g, 44.7%, white: mp $345\text{--}346^\circ C$; 1H NMR (DMSO- d_6) δ 0.97 (t, 3H, $J = 7.1$), 1.41 (m, 2H, $J = 7.2$), 1.65 (m, 2H, $J = 7.1$), 3.45 (2H), 7.14 (s, 1H), 7.61 (4H), 7.85 (d, 1H, $J_o = 8.5$), 8.04 (d, 1H, $J = 8.7$), 8.1 (3H), 8.75 (dd, 1H, $J_o = 8.7$, $J_m = 2$), 8.77 (d, 1H, $J = 2$), 9.0 (br s, 1H), 9.42 (br s, 1H), 9.76 (br s, 1H); MS (ESI) m/z 437 (M+1, 100); Anal. ($C_{27}H_{24}N_4O_2 \cdot 2HCl \cdot 0.5C_2H_5OH \cdot 0.25HOH$) C, H, N.

5.12.8. 6-[2-(5(6)-Cyclohexylamidinobenzimidazolyl)]-2-phenyl-4H-1-benzopyran-4-one hydrochloride (23). Yield 0.31 g, 34.4%, white: mp $>340^\circ C$; 1H NMR (DMSO- d_6) δ 1.36–2.0 (m, 10H), 3.78 (d, 1H), 7.14 (s, 1H), 7.62 (4H), 7.84 (d, 1H, $J_o = 8.5$), 8.04 (d, 1H, $J = 8.6$), 8.08 (s, 1H), 8.14 (m, 2H), 8.75 (d, 1H, $J_o = 8.7$, $J_m = 2.1$), 8.95 (d, 1H, $J = 2$), 9.11 (br s, 1H), 9.43 (br s, 1H), 9.55 (1H); MS (ESI) m/z 463 (M+1, 100); Anal. ($C_{29}H_{26}N_4O_2 \cdot 2HCl \cdot 0.25C_2H_5OH \cdot 2.3HOH$) C, H, N.

5.12.9. 6-[2-(5(6)-Benzylamidinobenzimidazolyl)]-2-phenyl-4H-1-benzopyran-4-one hydrochloride (24). Yield 0.345 g, 40.6%, white colour: mp $340\text{--}341^\circ C$; 1H NMR (DMSO- d_6) δ 4.77 (d, 2H), 7.13 (s, 1H), 7.35–7.62 (m, 8H), 7.72 (dd, 1H), 7.87 (d, 1H, $J_o = 8.5$), 8.0 (d, 1H, $J = 8.6$), 8.15 (m, 2H), 8.19 (s, 1H), 8.76 (dd, 1H, $J_o = 8.6$, $J_m = 1.9$), 8.95 (d, 1H, $J = 2$), 9.31 (s, 1H), 9.68 (s, 1H), 10.4 (t, 1H, $J = 4.9$); MS (ESI) m/z 471 (M+1, 100); Anal. ($C_{30}H_{22}N_4O_2 \cdot 2HCl \cdot 0.5C_2H_5OH$) C, H, N.

5.12.10. 2-Phenyl-6-[5(6)-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-1H-benzimidazol-2-yl]-4H-1-benzopyran-4-one hydrochloride (25). Yield 0.48 g, 62%, white: mp $>340^\circ C$; 1H NMR (DMSO- d_6) δ 2.01, 2.48 and 3.52 (m, 6H), 7.13 (s, 1H), 7.63 (m, 3H), 7.68 (d, 1H, $J = 8.4$), 7.84 (d, 1H,

$J = 8.7$), 8.04 (d, 1H, $J = 8.8$), 8.14 (m, 3H), 8.79 (dd, 1H, $J_o = 8.5$, $J_m = 2$), 8.95 (d, 1H, $J = 2.1$), 10.1 (s, 2H); MS (ESI) m/z 421 (M+1, 100); Anal. ($C_{26}H_{20}N_4O_2 \cdot 2HCl \cdot 1.2HOH$) C, H, N.

5.13. 6-[2-(5(6)-Amidinobenzimidazolyl)]-2-[4-(5(6)-amidinobenzimidazolyl)phenyl]-4H-1-benzopyran-4-one (27)

This compound was prepared by the general method of **14** with **26** (1 mmol), 3,4-diamino-benzamidine HCl·0.5-HOH (2 mmol), and 1,4-benzoquinone (2 mmol) in EtOH (20 mL) by refluxing for 24 h, yield 0.13 g, 20.2%, light yellow coloured: mp $>350^\circ C$; 1H NMR (DMSO- d_6) δ 7.03 (s, 1H), 7.41 (m, 3H), 7.54 (m, 3H), 7.82 (d, 1H, $J = 8.6$), 8.1 (m, 6H), 8.45 (d, 2H, $J = 8.2$), 8.68 (d, 1H, $J = 8.2$), 8.91 (s, 1H); MS (FAB) m/z 538.8 (M⁺); Anal. ($C_{31}H_{22}N_8O_2 \cdot 2HOH \cdot 1.5C_2H_5OH$) C, H, N.

5.14. 6-[2-(5(6)-Isopropylamidinobenzimidazolyl)]-2-[4-(5(6)-isopropylamidinobenzimidazolyl)phenyl]-4H-1-benzopyran-4-one hydrochloride (28)

This compound was prepared by the general method of **14** with **26** (1 mmol), 3,4-diamino-*N*-isopropylbenzamide HCl·0.5HOH (2 mmol), and 1,4-benzoquinone (2 mmol) in EtOH (20 mL) by refluxing for 24 h, yield 0.21 g, 25.9%, grey coloured (base colour is yellow): mp $>350^\circ C$; 1H NMR (DMSO- d_6) δ 1.35 (d, 12H, $J = 5.49$), 4.13 (m, 2H), 7.22 (s, 1H), 7.57 (t, 2H, $J = 8.1$), 7.78 (t, 2H, $J = 8.75$), 7.99 (d, 1H, $J = 8.8$), 8.09 (d, 2H, $J = 8.4$), 8.29 (d, 2H, $J = 8.6$), 8.51 (d, 2H, $J = 8.4$), 8.72 (dd, 1H, $J_o = 8.35$, $J_m = 1.9$), 8.88 (d, 1H, $J = 2$), 9.1 (s, 1H), 9.47 (s, 1H), 9.6 (s, 1H); MS (FAB) m/z 623.6 (M+1); Anal. ($C_{37}H_{34}N_8O_2 \cdot 4HCl \cdot HOH \cdot 0.5C_2H_5OH$) C, H, N.

5.15. 6-Bromomethyl-2-methyl-4H-1-benzopyran-4-one (31)

A mixture of **30** (0.8 g, 4.6 mmol) and *N*-bromosuccinimide (0.82 g, 4.6 mmol) in CCl_4 (20 mL) was heated at reflux overnight. The mixture was filtered while it was hot and CCl_4 was evaporated. The solid residue was crystallized from EtOAc-*n*-hexane, mp $115\text{--}121^\circ C$, a second time crystallization from EtOAc gave pure cubic crystal compound, yield (0.55 g, 47.4%): mp $139\text{--}140^\circ C$ lit²¹ $132^\circ C$; 1H NMR (DMSO- d_6) δ 2.4 (s, 3H), 4.86 (s, 2H), 6.26 (s, 1H), 7.59 (d, 1H, $J = 8.6$), 7.83 (dd, 1H, $J_o = 8.7$, $J_m = 2.2$), 8.08 (d, 1H, $J = 2.1$); MS (ESI) m/z 253 and 255 (M+1, 8), 173 (100); Anal. ($C_{11}H_9BrO_2$) C, H, N.

5.16. 2-Methyl-4H-1-benzopyran-4-one-6-carboxyaldehyde (32)

A mixture of **31** (0.55 g, 2.17 mmol) and hexamethylene-tetramine (2.2 g, 15.7 mmol) in 10 mL of acetic acid (50%) was heated at reflux for 2 h. HCl acid (5 mL, 50%) was added and refluxing was continued for 0.5 h. The reaction mixture was diluted with water and saturated with NaCl, then extracted with EtOAc. The organic layer was evaporated and the residue was crystallised

from EtOH–water, yield (0.28 g, 68.6%): mp 171–172 °C; ^1H NMR (CDCl_3) δ 2.43 (s, 3H), 6.36 (s, 1H), 7.78 (d, 1H, $J = 8.6$), 8.21 (dd, 1H, $J_o = 8.5$, $J_m = 2$), 8.57 (d, 1H, $J = 1.9$), 10.1 (s, 1H); MS (ESI) m/z 189 (M+1); Anal. ($\text{C}_{11}\text{H}_8\text{O}_3$) C, H.

5.17. 6-[2-(5(6)-Isopropylamidinobenzimidazolyl)]-2-methyl-4H-1-benzopyran-4-one hydrochloride (33)

This compound was prepared by the general method of **14** with **32** as a starting material, yield 0.29 g, 42.9%, light pink: mp >320 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 1.36 (d, 6H, $J = 6.4$), 2.47 (s, 3H), 4.12 (m, 1H, $J = 6.2$), 6.38 (s, 1H), 7.71 (dd, 1H, $J_o = 6.8$, $J_m = 1.7$), 7.87 (d, 1H), 7.90 (d, 1H), 8.14 (d, 1H, $J_m = 1.1$), 8.64 (dd, 1H, $J_o = 8.7$, $J_m = 2.2$), 8.92 (d, 1H, $J = 2.19$), 9.17 (s, 1H), 9.55 (s, 1H), 9.7 (d, 1H); MS (CI) m/z 361 (M+1); Anal. ($\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_2 \cdot 2\text{HCl} \cdot \text{HOH}$) C, H, N.

5.18. 6-[2-(5(6)-Butylamidinobenzimidazolyl)]-2-methyl-4H-1-benzopyran-4-one hydrochloride (34)

This compound was prepared by the general method of **14** with **32** as a starting material, yield 0.21 g, 30%, light grey: mp 305–309 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 0.94 (t, 3H, $J = 7.3$), 1.43 (m, 2H, $J = 7.2$), 1.66 (m, 2H, $J = 7.2$), 2.48 (s, 3H), 3.45 (q, 2H, $J = 7$), 6.34 (s, 1H), 7.70 (d, 1H), 7.82 (d, 1H, $J = 8.4$), 7.85 (d, 1H), 8.1 (s, 1H), 8.67 (d, 1H, $J = 8.7$), 8.89 (s, 1H), 9.01 (br s, 1H), 9.46 (br s, 1H), 9.76 (br s, 1H); MS (ESI) m/z 374 (M+1); Anal. ($\text{C}_{22}\text{H}_{22}\text{N}_4\text{O}_2 \cdot 2\text{HCl} \cdot 1.2\text{HOH}$) C, H, N.

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