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Convergent synthesis and biological evaluation of 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins

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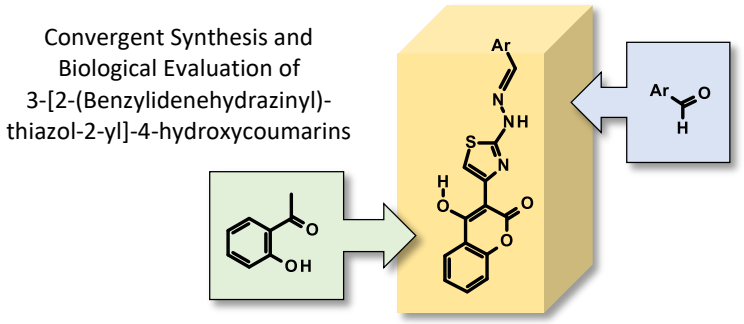
Abstract

A convenient synthesis of a small library of 4-hydroxycoumarin derivatives, containing thiazole and benzylidenehydrazine moieties has been achieved, using 2-hydroxyacetophenone and aryl- and heteroaryl aldehydes as starting materials. *In vitro* biological studies revealed that some of the compounds exhibited modest activities, *viz.;* anti-malarial (65% PLDH viability), anti-trypanosomal (52% *T.b.brucei* viability), anti-mycobacterial (Visual MIC90 5.63 μM) and anti-bacterial (restricting *S.aureus* metabolic activity after 6 h).



Keywords: 4-Hydroxycoumarin, thiazole, benzylidenehydrazine, synthesis, biological studies

Introduction

Both naturally occurring and synthetic coumarin derivatives have been observed to exhibit a wide range of biological activities.[1-4] Examples of naturally occurring coumarin derivatives include the antibiotic, novobiocin **1**,[5] and the highly toxic aflatoxins produced as fungal metabolites and illustrated by the fused-ring derivative, aflatoxin B_1 **2**.[6] Recently reported synthetic coumarin derivatives of medicinal interest include *inter alia*: anti-mycobacterial hydrazinyl thiazolyl derivatives, such as compound **3**;[7] non-steroidal anti-inflammatories, such as cloricromine **4**;[8] and anti-bacterial coumarin-triazole conjugates, such as compound **5** which exhibited a minimum MRSA inhibitory concentration of 4 μg/mL.[9]

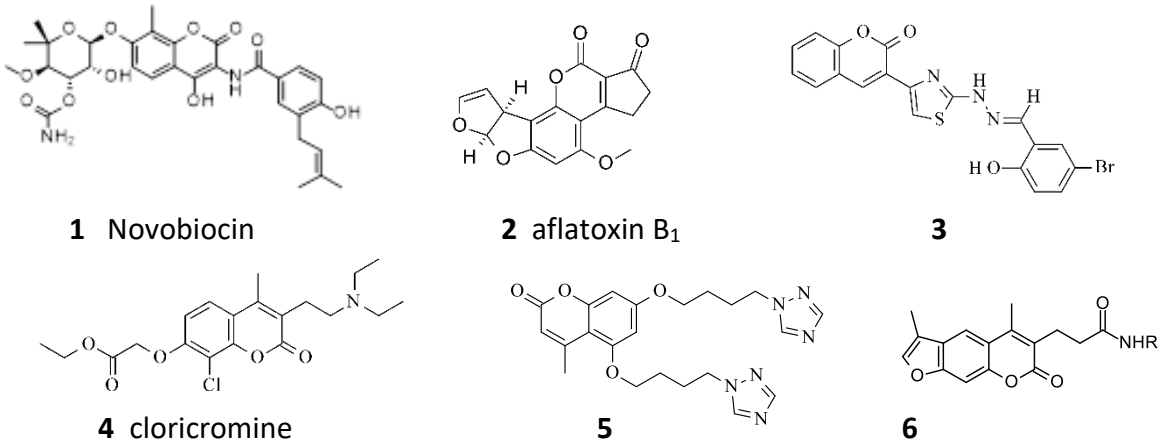


Figure 1. Structures of biologically active naturally occurring and synthetic coumarin derivatives.

Classic approaches to coumarin synthesis have included the Perkin, Knoevenagel and Pechmann and Wittig reactions.[10] In our group, we have pioneered applications of Morita-Baylis-Hillman (MBH) methodology in the construction of coumarin derivatives[11,12] and have reported the synthesis and *in vitro* biological evaluation of: novel furocoumarins **6** as potential HIV-1 IN inhibitors;[13] and coumarin-AZT conjugates.[14,15] as potential HIV-1 PR and dual-action HIV-1 PR/RT inhibitors. The 4-hydroxycoumarin motif is present in many biologically active compounds and, in this communication, we report on the preparation and bioassay of a series of 4-hydroxy analogues of the hydrazinyl thiazolyl derivative **3** reported by Arshad *et al*.[7]

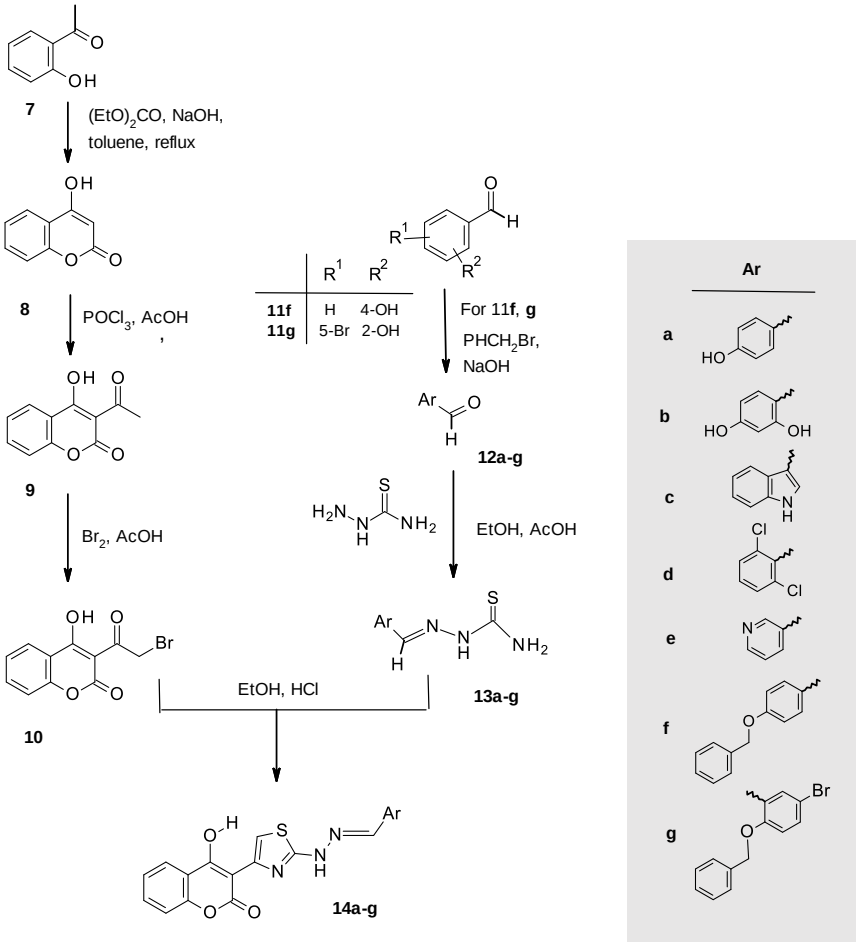
Results and Discussion

Synthesis of 3-[2-(3-[2-(benzylidinehydrazinyl)thiazol-4-yl]-yl]-4-hydroxycoumarins 14a-g

The title compounds were designed to incorporate, within a single hybrid structure, 4-hydroxycoumarin, thiazole and hydrazinyl moieties. It was hoped that MBH methodology, which we have successfully developed to access various benzannulated heterocyclic systems,[16] could be extended to generate the critical 4-hydroxycoumarin platform. Unfortunately, our efforts to access 4-hydroxycoumarins using MBH methodology were unsuccessful, and attention was turned to the procedure reported by Zhao *et al*.[17] Thus, in the first arm of the convergent synthesis, summarised in Scheme 1, reaction of 2-hydroxyacetophenone **7** with diethyl carbonate and sodium hydride in refluxing toluene afforded 4-hydroxycoumarin **8** in 69% yield. The 3-acetylated derivative **9** was obtained by following a method described by Sukdolak *et al*.; [18] which involved heating a

mixture of the substrate **8**, $POCl_3$ and acetic acid; increasing the reported duration of the reaction from 30 min. to 1 hour gave compound **9** in excellent yield (90%). Treatment of 2-acetyl-4-hydroxycoumarin **9** with bromine in acetic acid at 100° C afforded the α -brominated derivative **10**.

Following the approach used by Arshad et al., [7] the second arm of the convergent synthesis (Scheme 1) of the 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins **14a-g** involved preparation of the series of critical thiosemicarbazon**e** derivatives **13a-g** by refluxing ethanolic solutions of the substituted benzaldehydes **12a-g** and thiosemicarbazide to which a trace of 2N-HCl had been added. Most of the substituted benzaldehydes (**12**) were commercially available, but compounds **12f** and **12g** were specially prepared by benzylating the phenolic groups of the benzaldehyde precursors **11f** and **11g.** The thiosemicarbazon**e**s **13a-g** were isolated in satisfactory yields (65 – 76%).



Scheme 1. Convergent synthesis of 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins **14a-g.**

The targeted 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxy-coumarins **14a-g** were obtained in 62-87% yield by reacting 3-(2-bromoacetyl)-4-hydroxycoumarin **10** with the thiosemicarbazone derivatives **13a-g.** The products, which were isolated in high purity, as evidenced by NMR analysis, are all novel and were fully characterised.

Biological activity and computer docking studies

The 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins **14a-g** were subjected to anti-malarial, trypanocidal, cytotoxicity, anti-mycobacterial and anti-bacterial studies. The bioassay protocols are detailed and the results are tabulated in the Supporting Material file. The compounds generally exhibited weak anti-malarial and trypanocidal activity with the most active exhibiting, at best, only modest activity (**14a**: 65.3% PLDH viability: and **14b**: 52.0% *T.b.brucei* viability). Cytotoxicity, as determined at 20 µM against HeLa cells, ranged from 74.9 to 100% cell viability. Two of the compounds showed interesting anti-mycobacterial potential with respective visual and calculated MIC_{90} values of 15.63 and 31.25 µM for **14a** and and 31.25 and 15.63 µM for **14c**. In preliminary anti-bacterial assays conducted at Rhodes (*CCBR*), compounds **14a,c,d,e,g** proved inactive against *E.coli* cells but consistently active against *S.aureus*, with compound **14d** restricting metabolic activity to as little as 31% after 6h. Interestingly, parallel cell growth assays conducted at the University of Cape Town (*H3-D*) revealed no activity for these ligands against *S.aureus* after 16 h – raising the possibilities of rapid ligand metabolism, followed by pathogen recovery, or an initial delay in the implementation of the pathogen resistance mechanisms.

In silico docking studies of the synthetic ligands **14a**-**14g** in relevant enzyme structures were conducted using Schrödinger software.[19] Protein structures were obtained from the Protein Data Bank (5FRN[20] for HIV-1 integrase; 1YT9[21] and 1ZP8[22] for HIV-1 protease; 1T2C[23] and 1V0O[24] for *P.falciparum*; 3FWN[25] for *T.b.brucei*; and 4BFW[26] for *M.tuberculosis*). The binding affinities of ligands **14a-g** docked into the *T.b.brucei* enzyme structure 4FWN[25] ranged from -3.240 kcal/mol (**14d**) to -5.337 kcal/mol (**14b**). Compounds **14a** and **14b**, which exhibited significant activity against *T.b. brucei in vitro*, are among the three best ligands *in silico*. Interestingly, compound **14b** exhibited the best activity in both the *in vitro T.b.brucei* enzyme bioassay and the *in silico* 4FWN[25] docking analysis – results indicative of its potential as an anti-trypanosomal agent. In general, the binding affinities exhibited by the ligands (**14)** in their docking in the *M.tb* 4BFW[26] enzyme structure suggest their potential as lead compounds in the development of novel anti-mycobacterial agents. Only one of the ligands (**14d**) had a binding affinity weaker than -5.7 kcal/mol. Compound **14a**, on the other hand, exhibited the strongest binding affinity (-7.479 kcal/mol) – consistent with having the highest *in vitro Mtb* activity.

Conclusions

A small library of novel 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins, which contain medicinally significant 4-hydroxycoumarin, thiazole and hydrazinyl motifs, has been successfully prepared. The compounds have been fully characterised, and *in silico* docking studies and/or *in vitro* biological evaluation have suggested the trypanocidal, anti-mycobacterial and antibacterial (against *S.aureus*) activity of some of these compounds.

Experimental Section

General. Chemicals were purchased from Sigma-Aldrich and used without further purification. NMR spectra were recorded on a Bruker Biospin 600 MHz NMR spectrometer and chemical shifts were calibrated relative to the residual proton signal in DMSO-d_6 (2.5 ppm). IR Spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with a diamond window. Melting points were determined using a Stuart SMP30 apparatus

apparatus and are uncorrected. HRMS analyses were conducted at Rhodes University and by the Central Analytical Facilities Unit at the University of Stellenbosch.

The Supporting Information File contains: experimental methods and characterisation data for synthesised compounds; NMR spectra for new compounds; *in vitro* bioassay and *in silico* docking protocols; and *in silico* docking data.

The synthesis of compounds **13a-f** have been reported elsewhere.[27-29] Benzylation of the phenolic substrates **11f,g** to access compounds **12f,g** was effected following the method reported by Olomola *et al*.[30]

***N*^{1}-[2-(benzyloxy)-5-bromobenzylidene]-*N*^{2}-(thiocarbamoyl)hydrazine (13g).** A mixture of 2-(benzyloxy)-5-bromobenzaldehyde 0.58 g, 2 mmol), thiosemicarbazide (0.19, 2.1 mmol) and one or two drops of 2N-HCl was boiled under reflux in ethanol for at least 1.5 h. after which the resulting precipitate was filtered off and washed with several small volumes of methanol, filtered and air dried to afford *N*^{1}*-[2-(benzyloxy)-5-bromobenzylidene]-N*^{2}-(*thiocarbamoyl)hydrazine* **13g** as a pale yellow solid (0.14 g, 30%), mp 160-161 °C); [HRMS: *m/z* calculated for $C_{15}H_{15}BrN_3OS$ (MH^+) 364.0119. Found 364.0115]; ν_{max}/cm^{-1} 3431 and 3297 (N-H); δ_H (600 MHz; DMSO-d_6) 5.17 (2H, s, $PhCH_2$), 7.14 (1H, d, *J* 8.6 Hz, ArH), 7.30-7.37 (1H, m, ArH), 7.39-7.44 (2H, m, ArH), 7.46-7.52 (3H, m, ArH), 8.20 (2H, d, *J* 17.2 Hz, NH_2), 8.36 (1H, s, ArH), 8.46 (1H, s, 1'-CH), 11.53 (1H, s, NH); δ_C (150 MHz; DMSO-d_6) 69.9 ($PhCH_2$), 113.2, 115.5, 125.0, 127.4, 127.9, 128.2, 128.5, 133.3 and 136.4 (Ar-C), 136.5 (C=N), 155.9 (Ar-C), 178.0 (C=S).

T**he synthesis of the 3-[2-(benzylidenehydrazinyl)thiazol-4-yl]-4-hydroxycoumarins (14)** is illustrated by the following example. A mixture of 3-(2-bromoacetyl)-4-hydroxycoumarin **10** (0.071 g, 0.25 mmol), the hydrazine derivative **13a** (0.051 g, 0.26 mmol) and one or two drops of 2N-HCl was refluxed in ethanol for at least 2 hours, after which the resulting precipitate was filtered off and washed with methanol and then dried to afford coumarin **14a** as a white solid (0.073 g, 77%), mp 250 °C (decomp.); [HRMS: *m/z* calculated for $C_{19}H_{14}N_3O_4S$ (MH^+) 380.0705. Found 380.0705]; ν_{max}/cm^{-1} 1671 (C=N); δ_H (600 MHz; DMSO-d_6) 6.84 (2H, d, *J* 8.6 Hz, ArH), 7.35-7.39 (2H, m, ArH), 7.51 (1H, s, NN=CH), 7.53 (2H, d, ArH), 7.64 (1H, t, *J* 7.6 Hz, ArH), 7.94 (1H, d, *J* 7.3 Hz, ArH), 7.99 (1H, s, 5''-CH), 9.96 (1H, 4'-OH), 12.36 (1H, s, NH) and 15.82 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.0 (C-3), 104.6, 115.8, 116.1, 116.2, 123.9, 124.2, 124.9, 128.5, 132.8, 142.5, 144.4, 152.0 and 159.3 (Ar-C), 160.0 (C=O), 164.4 (C-OH) and 166.9 (C-2'').

3-{[2-(2,4-Dihydroxybenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin (**14b).** Yellow solid (0.074 g, 75%), mp 270 °C (decomp.); [HRMS: *m/z* calculated for $C_{19}H_{14}N_3O_5S$ (MH^+) 396.0654. Found 396.0662]; ν_{max}/cm^{-1} 1665 (C=N); δ_H (600 MHz; DMSO-d_6) 6.35 (2H, d, *J* 6.4 Hz, ArH), 7.38 (2H, t, *J* 8.1 Hz, ArH), 7.48 (1H, d, *J* 9.2 Hz, ArH), 7.51 (1H, s, NN=CH), 7.65 (1H, t, *J* 7.8 Hz, ArH), 7.95 (1H, d, *J* 7.9 Hz, ArH), 8.27 (1H, s, 5''-CH), 9.90 (1H, 4'-OH), 10.10 (1H, 6'-OH), 12.35 (1H, s, NH) and 15.83 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.0 (C-3), 102.5, 104.3, 108.1, 111.3, 116.1, 116.2, 123.9, 124.2, 128.2, 132.8, 142.5, 142.9, 152.0, 158.0 and 160.0 (Ar-C), 160.6 (C=O), 164.4 (C-OH) and 166.5 (C-2'').

4-Hydroxy-3-{[2-(1*H*-indol-3-yl)hydrazinyl]thiazol-4-yl}coumarin (14c). White solid (0.085 g, 84%), mp 240 °C (decomp.); [HRMS: *m/z* calculated for $C_{21}H_{15}N_4O_3S$ (MH^+) 403.0865. Found 403.0876]; ν_{max}/cm^{-1} 1696 (C=O); δ_H (400 MHz; DMSO-d_6) 7.19-7.26 (2H, m, ArH), 7.36-7.42 (2H, m, ArH), 7.48 (1H, dd, *J* 6.2, 2.3 Hz, ArH), 7.52 (1H, s, NN=CH), 7.66 (1H, t, *J* 7.6 Hz, ArH), 7.86 (1H, d, *J* 2.8 Hz, ArH), 7.98 (1H, d, *J* 8.2 Hz, ArH), 8.24 (1H, d, *J* 6.7 Hz, ArH), 8.34 (1H, s, 5''-CH), 11.63 (1H, s, 3'-NH), 12.33 (1H, s, NH) and 16.06 (1H, s, 4-OH); δ_C (100 MHz; DMSO-d_6) 95.6 (C-3), 103.5, 111.2, 112.1, 116.2, 116.5, 120.8, 121.6, 122.8, 123.9, 124.0, 124.1, 130.8, 132.7, 137.2, 142.1, 142.2 and 152.0 (Ar-C), 160.1 (C=O), 164.4 (C-OH) and 166.7 (C-2'').

3-{[2-(2,6-Dichlorobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin (14d). White solid (0.08 g, 74%), mp 275 °C (decomp.); [HRMS: *m/z* calculated for $C_{19}H_{12}Cl_2N_3O_3S$ (MH^+) 431.9976. Found 431.9963]; ν_{max}/cm^{-1}

1676 (C=N); δ_H (600 MHz; DMSO-d_6) 7.36-7.41 (3H, m, ArH), 7.55 (2H, d, *J* 8.1 Hz, ArH), 7.60 (1H, s, NN=CH), 7.66 (1H, t, *J* 7.7 Hz, ArH), 7.94 (1H, d, *J* 7.5 Hz, ArH), 8.31 (1H, s, 5''-CH), 12.80 (1H, s, NH) and 15.43 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.4 (C-3), 106.4, 115.6, 116.2, 123.8, 124.3, 129.1, 129.5, 130.9, 132.9, 133.7, 138.3, 142.8 and 151.9 (Ar-C), 159.8 (C=O), 163.6 (C-OH) and 166.9 (C-2'').

4-Hydroxy-3-{[2-(pyridin-3-yl)hydrazinyl]thiazol-4-yl}coumarin (14e). Yellow solid (0.079 g, 87%), mp 290 °C (decomp.); [HRMS: *m/z* calculated for $C_{18}H_{13}N_4O_3S$ (MH^+) 365.0716. Found 365.0708]; ν_{max}/cm^{-1} 1666 (C=N); δ_H (600 MHz; DMSO-d_6) 7.35-7.40 (2H, m, ArH), 7.62-7.67 (2H, m, ArH), 7.91-7.95 (2H, m, ArH), 8.18 (1H, s, NN=CH), 8.59 (1H, d, *J* 8.2 Hz, ArH), 8.80 (1H, d, *J* 5.0 Hz, ArH), 9.06 (1H, s, 5''-CH), 13.00 (1H, s, NH) and 15.34 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.4 (C-3), 106.7, 115.7, 116.3, 123.8, 124.4, 126.5, 132.6, 133.0, 138.0, 139.2, 142.5, 142.9, 144.1 and 151.9 (Ar-C), 159.9 (C=O), 163.7 (C-OH) and 166.6 (C-2'').

3-{[2-(4-Benzyloxybenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin (14f). White solid (0.085 g, 72%), mp 285-287 °C; [HRMS: *m/z* calculated for $C_{26}H_{20}N_3O_4S$ (MH^+) 470.1175. Found 470.1181]; ν_{max}/cm^{-1} 1671 (C=N); δ_H (600 MHz; DMSO-d_6) 5.13 (2H, s, CH_2), 7.07 (2H, d, *J* 7.9 Hz, ArH), 7.32-7.35 (1H, m, ArH), 7.36-7.42 (4H, m, ArH), 7.45 (2H, d, *J* 7.1 Hz, ArH), 7.54 (1H, s, NN=CH), 7.60-7.67 (3H, m, ArH), 7.94 (1H, d, *J* 7.3 Hz, ArH), 8.02 (1H, s, 5''-CH), 12.43 (1H, s, NH) and 15.77 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 69.4 (CH_2), 96.1 (C-3), 104.9, 115.2, 116.0, 116.2, 123.8, 124.2, 126.6, 127.8, 128.0, 128.3, 128.5, 132.8, 136.7, 142.5, 143.8, 151.9 and 159.8 (Ar-C), 159.9 (C=O), 164.2 (C-OH) and 166.9 (C-2'')

3-{[2-(2-Benzyloxy-5-bromobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin (14g). White solid (0.085 g, 62%), mp 255-257 °C; [HRMS: *m/z* calculated for $C_{26}H_{19}BrN_3O_4S$ (MH^+) 548.0280. Found 548.0297]; ν_{max}/cm^{-1} 1675 (C=N); δ_H (600 MHz; DMSO-d_6) 5.13 (2H, s, CH_2), 7.08 (1H, d, *J* 8.8 Hz, ArH), 7.30-7.35 (2H, m, ArH), 7.38 (2H, q, *J* 7.6 Hz, ArH), 7.44 (2H, t, *J* 7.3 Hz, ArH), 7.50 (2H, d, *J* 7.5 Hz, ArH), 7.52 (1H, s, NN=CH), 7.60 (1H, t, *J* 7.6 Hz, ArH), 7.77 (1H, s, ArH), 7.88 (1H, d, *J* 7.7 Hz, ArH), 8.24 (1H, s, 5''-CH), 12.54 (1H, s, NH) and 15.49 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 70.1 (CH_2), 96.2 (C-3), 105.5, 112.8, 115.4, 115.7, 116.1, 123.7, 124.1, 124.4, 126.9, 127.8, 128.1, 128.6, 132.7, 133.0, 136.4, 137.0, 142.7, 151.8 and 155.4 (Ar-C), 159.8 (C=O), 163.6 (C-OH) and 166.6 (C-2'').

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Supplementary Material

Bioassay and *in silico* docking protocols and data, and NMR spectra for all new compounds are provided in the Supporting Information file.

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