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Synthesis and biological evaluation of bis-N^2,$N^{2\prime}$-(4-hydroxycoumarin-3-yl) ethylidene]-2,3-dihydroxysuccinodihydrazides

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ABSTRACT

A series of N^2,$N^{2\prime}$-bis[4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccino-hydrazides, containing 4-hydroxycoumarin, hydrazine and tartaric acid moieties, have been prepared and examined for possible biological activity. Several of these compounds exhibit promising HIV-1 integrase inhibition (IC_{50} = 3.5 μM), and anti-*T. brucei* (32% viability) and anti-mycobacterial (Visual MIC90 = 15.63 μM) activity.

Numerous coumarin derivatives have been found to be present as secondary metabolites in bacteria, fungi and plants, thus prompting research into their isolation and the study of their biological activities.[1] Such derivatives exhibit wide varieties of medicinal properties, including anti-parasitic, anti-HIV, anti-bacterial, as well as serine protease, cholinesterase and lipoxygenase monoamine oxidase inhibitory activities.[2–4] The antibiotics, novobiocin (isolated from *Streptomyces niveus* and *Streptomyces spheroides*) and chartreusin (isolated from *Streptomyces chartreusis*) are examples of coumarin derivatives isolated from bacteria,[5] while aflatoxins are highly toxic, coumarin-containing, fungal metabolites found in *Aspergillus* species.[6]

Various biologically active coumarin derivatives have also been synthesised,[7] including: coumarin-2-carboxamides,[8] which exhibit anticancer activity; the 4-hydroxycoumarin derivative **1**, which exhibits HIV-1 protease (PR) inhibition activity;[9] and coumarin-containing hydrazine derivatives, such as compound **2**, which exhibit minimum inhibitory concentrations (MIC) of 15–17 μM against *M. tuberculosis*.[10] In their highly cited paper, Zhao *et al.*,[11] discuss coumarin-based HIV Integrase (IN) inhibitors. More recently, 4-hydroxycoumarin dimers[12] and coumarin-3-carbohydrazide derivatives[13] have been reported to exhibit HIV-1 IN inhibitory activities with IC_{50} values in the low micromolar and nanomolar ranges, respectively. In earlier studies, we have explored the synthesis and biological activity of various coumarin derivatives,[14–17] including furocoumarins as potential HIV-1 (IN) inhibitors[14] and coumarin-AZT conjugates **3** as potential dual-action HIV-1 PR and reverse transcriptase (RT) inhibitors.[17] We now report the synthesis and bioactivity of a series of 4-hydroxycoumarin derivatives generated by the conjunction of 4-hydroxycoumarin, hydrazine and tartaric acid moieties.

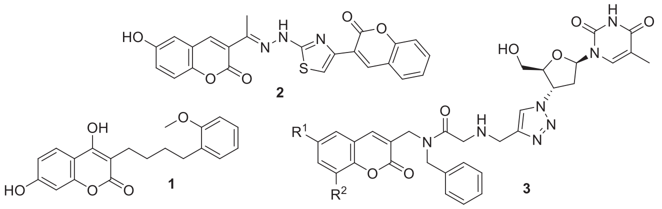

Fig. 1. Structures of synthetic, biologically active coumarin derivatives.

The wide spectrum of applications of coumarin derivatives has led to considerable interest in their synthesis and numerous methods have been developed. These include the classic Perkin, Knoevenagel and Pechmann syntheses as well applications of Morita-Baylis-Hillman

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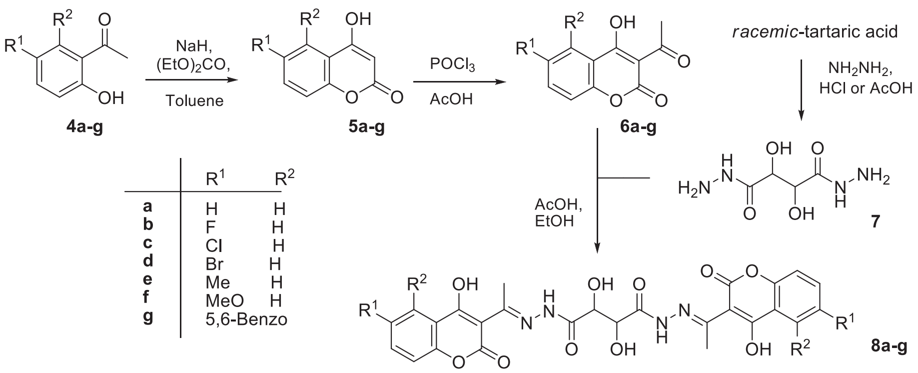


Scheme 1. Synthesis of bis-(4-hydroxycoumarinyl)succinohydrazides.

(MBH) methodology developed by our group.[18] However, our attempts to use MBH methodology to access the 4-hydroxycoumarins derivatives required in this study proved unsatisfactory and, consequently, the 4-hydroxycoumarins **5a–g** (Scheme 1) were prepared using the method reported by Zhao et al.[19] but with minor modifications, *viz.*, i) the use of dimethyl carbonate, in some cases, instead of diethyl carbonate; and ii) omitting recrystallisation from ethanol when NMR analysis indicated formation of the products in satisfactory purity. The 2-hydroxyacetophenones **4a–g** were reacted with either diethyl or dimethyl carbonate in the presence of sodium hydride to afford the corresponding 4-hydroxycoumarins **5a–g** in yields of 60–87%.

Acetylation of the 4-hydroxycoumarins **5a–g** was effected following the method described by Sukdolak *et al.*[20] It was observed, however, that the yields improved with longer reaction times (at least 1 h rather than the reported 30 min); moreover, the purity of the compounds was satisfactory after washing the precipitate with methanol thereby permitting omission of a crystallisation step. Thus, the 4-hydroxycoumarins **5a–g** were each reacted with $POCl_3$ in glacial acetic acid under reflux for at least 1 h, after which the resulting precipitates were filtered, washed with methanol and dried to give the respective products (**6**) in yields ranging from 41% to 90%.

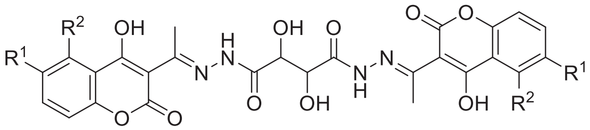
The 2,3-dihydroxysuccinodihydrazide **7,** required for the final step in the preparation of the title compounds **8a–g**, was obtained *via* acid (HCl or AcOH)-catalysed reaction of *racemic* diethyl tartrate with hydrazine hydrate in refluxing ethanol.[21] The resulting precipitate was recrystallised from methanol to give the desired dihydrazide **7** as a white solid, which was then reacted with the 3-acetylated 4-hydroxycoumarins **6a–g** in ethanol under reflux for at least 2 h (Scheme 1).[22] The precipitates formed in each reaction were filtered, washed with methanol and dried to give the required bis[4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinodihydrazides **8a–g** in high purity and in yields of 41%–58%.

The targeted products **8a–g** contain: i) the 4-hydroxycoumarin moiety present in the HIV-1 PR inhibitor **1**; ii) the hydroxyethylene peptide isosteres and overall C_2 symmetry characteristic of clinical HIV-1 PR inhibitors; and iii) the coumarin and hydrazine moieties present in the anti-mycobacterial derivative **2** and the recently reported coumarin-3-carbohydrazide HIV-1 IN inhibitors.[13] Compounds **8a–g** were thus screened for HIV-1 IN and PR inhibition potential, anti-tuberculosis (*M. tuberculosis*) activity and cytotoxicity (against HeLa cells). The opportunity was also taken to screen the compounds for anti-*Plasmodium falciparum* (*P. falciparum*) and anti-trypanosome (*T.b. brucei*) activity. The results of the bioassays are summarised in Table 1.

With the exception of the brominated derivative **8d**, all of the title compounds (**8**) showed some inhibition of HIV-1 IN – particularly, the parent system **8a** with an IC_{50} value of 3.5 μM. It is interesting to note that sequential introduction of a halogen substituent (R^1 = F, Cl, Br) into the unsubstituted aromatic rings in **8a** (R^1 = H) resulted in graded decreases in the HIV-1 IN inhibition (**8a**: 59.4% > **8b**: 35.4% > **8c**: 27.6% > **8d**: 0%) which appear to follow their increasing bulk *inversely* and their electronegativity *directly*. The apparent advantage conferred by the absence of additional substituents in the benzene ring is reflected in the HIV-1 inhibition activity of other 4-hydroxycoumarin derivatives.[11] Only one of the compounds (**8e**; 8.3% inhibition) proved

Table 1

Bioassay data for compounds **8a–g**, showing % inhibition of HIV-1 IN and PR, % viability of *P. falciparum*, *T.b. brucei* and HeLa cells at 20 μM and MIC_{90} values against *M. tuberculosis*.



8a-g

Compd.	R^1	R^2	% Yield	% HIV-1 IN inhibition[a]	% HIV-1 PR inhibition[b]	% *P. falciparum* viability[c]	% *T.b. brucei* viability[d]	% HeLa cell viability[e]	Visual MIC90 (μM)[f,g]	Calc. MIC90 (μM)[f,h]
8a	H	H	58	59.4[i]	0.0	90.7	42.7	78.4	> 125	> 125
8b	F	H	58	35.4	0.0	92.8	32.8	84.6	> 125	> 125
8c	Cl	H	45	27.6	0.0	93.8	44.6	100.0	31.25	54.25
8d	Br	H	42	0.0	0.0	100.0	100.0	100.0	15.63	18.91
8e	Me	H	41	26.4	8.3	100.0	100.0	85.3	> 125	> 125
8f	MeO	H	43	34.6	0.0	100.0	100.0	84.6	> 125	> 125
8g	5,6-Benzo		45	35.7	0.0	100.0	100.0	98.1	31.25	29.46
Control				100.0[a]	99.8[b]	–[c]	–[d]	–[e]	0.019[f]	0.007[f]

Controls: [a]chicoric acid; [b]ritonavir; [c]chloroquine: IC_{50} = 0.012 μM; [d]pentamidine: IC_{50} = 0.039 μM; [e]emetine: IC_{50} = 0.013 μM and [f]rifampicin. [g]Visual MIC90 7D 7H9 GLU CAS Tx. [h]Calculated MIC90 7D 7H9 GLU CAS Tx (μM). [i]IC_{50} = 3.5 μM.

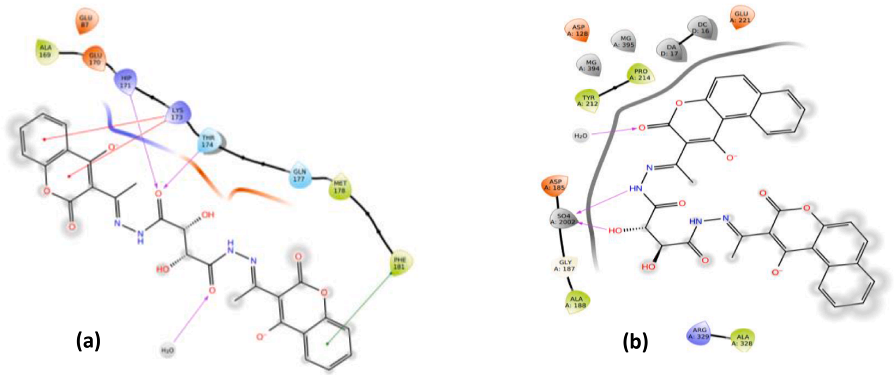


Fig. 2. Potential hydrogen-bonding interactions between amino acid residues in: a) the non-catalytic active-site of HIV-1 IN (PDB 4E1M) and compound **8a**; and b) the active-site of HIV-1 IN (PDB 5FRN) and compound **8g**.

to be active, albeit very weakly, against HIV-1 PR at a concentration of 20 μM.

The products **8a**–g were also evaluated for anti-parasitic activity at a concentration of 20 μM against both *P. falciparum* and *T.b. brucei* (which is responsible for sleeping sickness). The products showed little, if any, significant activity in the *P. falciparum* screen; compounds **8a**–**c**, however, clearly exhibited anti-trypanosomal activity, decreasing residual parasite *T.b. brucei* viabilities appreciably (**8a**: 43%; **8b**: 33%; and **8c**: 45%). When tested at a concentration of 20 μM against HeLa cells, none of the compounds **8a**–**g** exhibited significant cytotoxicity. As indicated earlier, coumarin-containing hydrazine derivatives (*e.g.*, compound **2**, Fig. 1) have been reported by Arshad et al.[13] to exhibit minimum inhibitory concentrations (MIC) in the 15–17 μM range against *M. tuberculosis*, and three of our compounds (**8c**, **8d** and **8g**) have also shown promising anti-mycobacterial potential with relatively low visual MIC_{90} (31.25, 15.63 and 31.25 μM, respectively) and calculated MIC_{90} values (54.25, 18.91 and 29.46 μM, respectively).

In silico docking studies were undertaken to assess the binding affinity of compounds **8a**–**g** to selected HIV-1 IN and PR, *P. falciparum*, *T. Brucei* and *M. tuberculosis* enzyme receptors, the detailed results of which are summarised in the Supporting Information file. Clear correlations between the *in silico* binding affinities and the *in vitro* data generally proved to be limited, but several instances are noteworthy. Compound **8a**, which exhibited the highest *in vitro* HIV-1 IN inhibition (Table 1) exhibited the best *in silico* binding affinity (−4.628 kcal/mol) in the non-catalytic active-site of HIV-1 IN crystal structure PDB 4E1M (illustrated in Fig. 2a) and the second best binding affinity (−6.215 kcal/mol) in the HIV-1 IN crystal structure PDB 5FRN. Interestingly, compound **8g**, which exhibited the second-highest *in vitro* HIV-1 IN inhibition, exhibited the *best* binding affinity (−6.794 kcal/mol) for 5FRN, but the *lowest* binding affinity (−0.163 kcal/mol) for 4E1M; the binding of this ligand in the active site of 5FRN is illustrated in Fig. 2b. The strong *in silico* HIV-1 PR 1YT9 binding affinities for all of the ligands **8a**–**g** (between −6.744 and −9.420 kcal/mol, compared with −7.309 kcal/mol for the control, ritonavir) are completely at odds with the *in vitro* data in Table 1! The corresponding *in silico* HIV-1 PR IZP8 binding affinities for the ligands **8a**–**g** are all significantly lower than that of ritonavir but are at least consistent with their general, *in vitro* inactivity.

Although compounds **8a**–**g** all exhibited very encouraging *in silico* binding affinities (relative to chloroquine) with three different *P. falciparum* enzyme structures, none of them exhibited significant *in vitro* anti-parasitic activity. The three compounds with the strongest *in vitro* anti-trypanosomal activity (**8a**–**c**) exhibit the weakest *in silico* binding affinities with the *T.b. brucei* enzyme structure! It is similarly difficult to draw any meaningful correlations between the *in vitro* anti-mycobacterial and the *in silico* enzyme-binding affinities of the title compounds.

In summary, it is apparent that the title compounds are readily accessible, exhibit insignificant cytotoxicity and some of them show promising HIV-1 IN inhibition (IC_{50} = 3.5 μM), anti-*T. brucei* activity (32% viability at 20 mM) and anti-mycobacterial potential (Visual MIC90 = 15.63 μM). Experimental data for the synthesised compounds, NMR spectra for all new compounds, the *in silico* docking protocols and the resulting binding affinities are provided in the Supporting Information file. Bioassay protocols have been reported previously.[23,24]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126911.

References

1. Revankar HM, Bukhari SNA, Kumar GB, Qin H-L. *Bioorg Chem.* 2017;71:146.
2. Vekariya RH, Patel HD. *Synth Commun.* 2014;44:2756.
3. Jameel E, Umar T, Kumar J, Hoda N. *Chem Biol Drug Des.* 2016;87:21.
4. Stefanachi A, Leonetti F, Pisani L, Catto M, Carotti A. *Molecules.* 2018;23:250.
5. Venugopala KN, Rashmi V, Odhav B. *Biomed Res Int.* 2013;2013:14.
6. Aoife L. *Curr Pharm Des.* 2004;10:3797.
7. Mohd ZH, Hasnah O, Mohamed AA, Mohamed JA. *Eur J Med Chem.* 2016;123:236.
8. Reddy NS, Gumireddy K, Mallireddigari MR, et al. *Bioorg Med Chem.* 2005;13:3141.
9. Lunney EA, Hagen SE, Domagala JM, et al. *J Med Chem.* 1994;37:2664.
10. Arshad A, Osman H, Bagley MC, et al. *Med Chem.* 2011;46:3788.
11. Zhao H, Neamati N, Hong H, et al. *J Med Chem.* 1997;40(2):242.
12. Mao PC-M, Mouscadet J-F, Leh H, Auclair C, Hsu L-Y. *Chem Pharm Bull.* 2002;50:1634.
13. Jesumoroti OJ, Faridoon, Mnkandhla D, Isaacs M, Hoppe HC, Klein R. *Med Chem Commun.* 2019;10:80.
14. Olomola TO, Mosebi S, Klein R, et al. *Bioorg Chem.* 2014;57:1.
15. Faridoon, Olomola TO, Tukulula M, Klein R, Kaye PT. *Tetrahedron.* 2015;71:4868.
16. Olasupo I, Rose NR, Klein R, Adams LA, Familoni OB, Kaye PT. *Synth Commun.* 2014;44(2):251.
17. Olomola TO, Klein R, Mautsa N, Sayed Y, Kaye PT. *Bioorg Med Chem.* 2013;21:1964–1971.

18. Faridoon, Olomola TO, Tukulula M, Klein R, Kaye PT. *Tetrahedron.* 2015;71:4868 and references cited therein.
19. Zhao P-L, Wang L, Zhu X-L, et al. *J Am Chem Soc.* 2010;132:185.
20. Sukdolak S, Solujić S, Manojlović N, Vuković N, Krstić LJ. *J Heterocycl Chem.* 2004;41:593.
21. Tian F-F, Jiang F-L, Han X-L, et al. *J Phys Chem B.* 2010;114:14842.
22. Shakdofa M. *Indian J Adv Chem Sci.* 2016;4:114.
23. Adeyemi CM, Faridoon R, Isaacs M, et al. *Bioorg Med Chem.* 2016;24:6131–6138.
24. Olawode EO, Tandlich R, Prinsloo E, et al. *Bioorg Med Chem Lett.* 2019;29:1572–1575.