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Evaluation of novel Hyphodermin derivatives as Glycogen Phosphorylase a inhibitors

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Abstract—The lipophilicity, permeability, solubility, polar surface area and 'rule-of-five' properties were assessed, using QikProp v2.5 (Schrödinger, Inc.) and ALOGPS 2.1 calculations, for 25 Hyphodermin derivatives. These compounds obeyed the 'rule-of-five', and the calculated physicochemical values were generally within desired limits. All compounds were tested against Glycogen Phosphorylase a (GPa). Four phenyl and benzyl substituted 2-oxo-hexahydro and tetrahydrobenzo[*cd*]indole carboxylic acids were identified as novel inhibitors of GPa with estimated IC_{50} values in the range 0.8–1.3 mM. Molecular modelling of these novel inhibitors was used to obtain the main structural features of this class of molecule for future structure–activity relationship studies.

1. Introduction

Glycogen Phosphorylase (GP) is a molecular therapeutic target for treating hyperglycaemia,[1–3] as it plays a crucial role in the formation of glucose-1-phosphate from hepatic glycogen stores. Of the known binding sites of GP, the majority of inhibitors target either the catalytic site[3] which binds glucose,[4,5] glycogen and analogues of these[6]; the allosteric (AMP) site which binds AMP, IMP, ATP and glucose-6-phosphate[1]; the purine nucleoside site, (an allosteric site also referred to as 'the inhibitor site') which is active towards caffeine,[7] purine derivatives and a range of heterocyclic compounds; or the indole site (another allosteric site) at the enzyme dimer interface which binds indoles.[8–10] Recently, a possible novel binding site on the surface of the protein located roughly 32 Å from any other binding site has been reported.[11] Most inhibitors of these sites have been screened *in vitro* with some inhibitors also screened in vivo. In the case of the indole inhibitors, GPa has been validated as a target in diabetic *ob*/*ob* mice.[12] Comprehensive reviews on a range of GP inhibitors have been reported elsewhere.[1,2,13,14] GP is heavily regulated by allosteric effectors. Allosteric manipulation of GP potentially reduces the need to target the highly conserved catalytic site, and allows for the exploration of structurally unique molecules as agents for regulatory control of GP. The design of biologically active molecules based on lead compounds from nature is a commonly used technique in drug design. Natural products provide biologically validated lead compounds, thus provide a higher success rate in the design of potent biologically active molecules.[15] Analogues of natural products are more likely to be developed into useful therapeutic agents, by the introduction of groups giving rise to more 'drug-like' molecules which may enhance binding strength and thus potency of the potential inhibitor. Current hypoglycaemia drugs have limited efficacy and thus there is a continued search for compounds that can improve current therapies for diabetes.

Hyphodermins A–H, a novel class of naphtho[1,2-*c*]furan-3,9-diones, isolated from a culture of Basidiomycete hyphoderma radula (WP 2184) obtained from the trunk of wild cherry tree in Wuppertal (Germany),[16] are reported to inhibit the interaction between GPa and PP-1.[16] This interaction indicates, although not confirmed, Hyphodermins A–H could potentially be allosteric interactors of GPa. In the present work, we chose

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Hyphodermins A, B and D (compounds **13**, **9** and **14**, respectively, Fig. 1) as possible lead compounds along with simple novel derivatives (compounds **1–8, 10–12 and 15–25**, Fig. 1) and sought to assess and validate these compounds as potential new inhibitors of GPa. Of particular interest are the lipophilicities, solubilities, polar surface areas and Lipinski correlations. The results of theoretical studies of compounds **1–25** are discussed. In addition, compounds **1–25** were screened against GPa to identify whether these compounds could indeed be classified as a potential new class of GPa inhibitor.

2. Results and discussion

2.1. Hyphodermin A, B and D (13, 9 and 14) derivatives

The choice of derivatives of Hyphodermins A, B and D (**13, 9** and **14**) was guided by synthetic accessibility. The hemiacetal moiety of **9**, **13** and **14** offers a number of possibilities for binding interactions with the enzyme and it was this moiety we choose to explore with selected derivatives. Anhydride **8** was an accessible synthetic intermediate that served as a molecular scaffold as it can be functionalised by ring opening with a range of nucleophiles. Notably, nucleophilic attack occurs exclusively at C1 of the anhydride **8**, as shown in the example of ester **15**. A representative view of the structure of **15** is shown in Figure 2. In this structure, the carboxylic acid group is almost co-planar with the phenyl ring (C3–C2–C21–O2 = 9.9(3)°) while the methyl ester group lies orthogonal to this ring (C2–C1–C11–O2 = 85.1(3°)). The carboxylic acid and methyl ester groups and the co-crystallized water molecules form an extensive intermolecular O–H…O hydrogen bonding network vis alternating $R_4^4(12)$ and $R_4^4(18)$ ring patterns (Fig. 2).

In consideration of the synthesis of a set of derivatives (compounds **15–25**), we replaced the ketone and alcohol

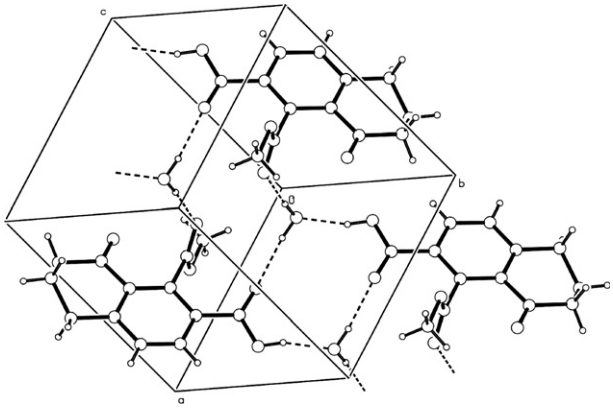

Figure 2. Representation of the H-bonding structure in **15**.

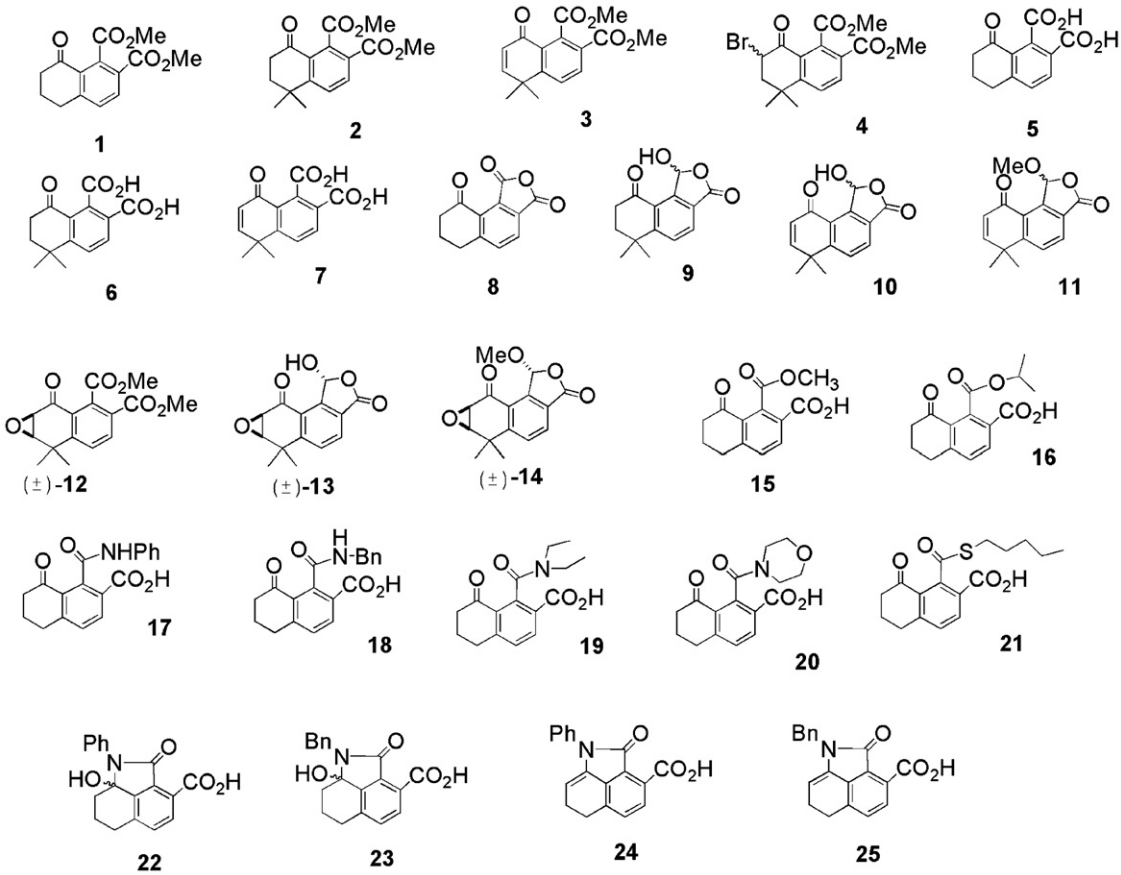

Figure 1. Structures of Hyphodermin A, B and D (**13**, **9** and **14**) and derivatives, compounds **1–8, 10–12** and **15–25.**

functional groups by more drug-like groups, such as amides (compounds **17**–**20** and **22**–**25**), that might increase the potency of synthetic derivatives. Other appropriate structural derivatives (**1**–**7**, **10**–**12**) were accessed during synthetic studies. Syntheses of compounds **1**–**25** are reported elsewhere.[17–19] All compounds investigated are shown in Figure 1. Compounds **1**–**25** were converted to their SMILES representations for QikProp v2.5 (Schrödinger, Inc.)[20] and ALOGPS 2.1[21,22] calculations.

2.2. Lipophilicity and permeability

In the absence of experimental data, prediction of significant physicochemical associated properties, such as lipophilicity, solubility and permeability,[23–27] in conjunction with synthetic work is important to aid determination of the best candidates for progression. It is common practice to use Log *P*, the partition coefficient between water and octanol, as a reliable indicator of the lipophilicity of (drug) molecules.[28] The computed Log *P* values (Table 1) were obtained using QikProp and the 'consensus' prediction[29] from ALOGPS 2.1 (which obtains the average from values calculated with ALOGPS 2.1, Pharma Algorithms Log *P*, Log *S* and pK_a, Actelion Log *P*, COSMOfrag Log *P*, Molinspiration Log *P*, KOWWIN Log *P* and XLOGP programs). The Log *P* values do not account for modifications in the hydrophobicity of ionizable compounds at varying pH. Theoretically predicted Log *D* values were not calculated as they are often not sufficiently accurate for practical usage,[30] and lipophilicity expressed as Log *D* has not given a satisfactory correlation with absorption as reported elsewhere.[31]

In Table 1, compounds **1**–**20**, and **22**–**25** have a predicted Log *P* < 5 in accordance with Lipinski's rules,[26,32] and another study which suggests a value of <3.5 for Log *P* is preferred for lead-like compounds.[33] In addition, a low Log *P* in the range of 0–3 is desirable to reduce toxicity and increase ease of formulation and bioavailability for optimal oral absorption. All compounds studied had Log *P* values within the desired range for optimal oral absorption, apart from compounds **21** and **25** (by both calculation methods) and compounds **8**, **13** and **14** (QikProp method), which had values generally 0.5 outside the desired range.

As another potential indication of permeability, the predicted apparent Caco-2 cell permeability values (QPPCaco) which are used as a model for the gut–blood barrier,[34] were obtained using QikProp (Table 1). QikProp predictions are for non-active transport. In Table 1, compounds **5**–**7** were predicted to have a QPPCaco < 25 nm/s suggestive of poor permeability, compounds **8–10, 13** and **15–25** were predicted to have a QPPCaco 25–500 nm/s suggestive of moderate permeability and compounds **1–4, 11, 12** and **14**, were predicted to have a QPPCaco > 500 nm/s suggestive of great permeability.

2.3. Solubility

An indication of the solubility of a compound (*S*) can be given by the Log *S* value. As reviewed elsewhere[35] an acceptable level of solubility is critical to permit dissolution and absorption. Absorption of a drug is usually very low if the calculated solubility is <0.00001 mg/L, as shown in a study of the rate-limiting steps of human oral absorption of 238 drugs.[36] As the experimental solubilities of the compounds **1–25** were not known, the Log *S* values were calculated using ALOGPS 2.1 and QikProp. Differences were observed between the Log *S*

Table 1. Calculated lipophilicities (ALOGPs, QPLogP), permeability (QPPCaco) and solubilities (ALOGpS, QPLogS) and for compounds **1–25**

Compound	ALOGPs	QPLogP	QPPCaco	ALOGpS	QPLogS	ALOGpS (g/L)	QPLogS (g/L)
1	1.80 (±0.63)	1.383	970.527	−3.14	−2.448	0.19	0.93
2	2.56 (±0.48)	1.952	973.757	−4.06	−3.153	0.03	0.20
3	2.82 (±0.36)	1.902	773.194	−4.11	−3.258	0.02	0.16
4	2.94 (±0.49)	2.608	1127.205	−4.96	−3.991	0.01	0.04
5	1.24 (±0.59)	1.016	7.057	−2.57	−2.060	0.63	2.04
6	1.93 (±0.59)	1.520	7.097	−3.02	−2.633	0.25	0.61
7	1.87 (±0.89)	1.489	6.628	−2.72	−2.582	0.49	0.68
8	1.60 (±0.57)	−0.605	281.939	−2.50	−0.266	0.68	117.08
9	1.45 (±0.77)	0.427	397.024	−2.11	−1.925	1.92	2.93
10	1.44 (±1.10)	0.397	360.624	−2.95	−1.930	0.28	2.87
11	2.15 (±0.75)	0.694	1153.318	−3.30	−1.172	0.13	17.36
12	1.68 (±0.52)	0.821	836.067	−3.33	−1.854	0.14	4.26
13	0.51 (±0.88)	−0.443	386.449	−1.36	−1.056	11.39	22.88
14	1.15 (±0.75)	−0.409	1221.994	−2.00	0.314	2.77	564.73
15	1.55 (±0.48)	1.328	89.321	−2.69	−2.426	0.50	0.93
16	2.34 (±0.52)	2.083	129.202	−3.06	−3.031	0.24	0.26
17	2.59 (±0.56)	2.476	137.432	−4.27	−3.533	0.05	0.09
18	2.55 (±0.81)	2.742	174.787	−4.47	−3.612	0.01	0.08
19	1.87 (±0.49)	1.992	182.846	−2.68	−2.608	0.60	0.71
20	0.74 (±0.51)	1.115	156.132	−2.13	−2.063	2.23	2.62
21	4.03 (±0.39)	3.136	150.242	−4.17	−3.890	0.02	0.04
22	2.27 (±0.53)	2.450	90.341	−3.28	−3.656	0.16	0.07
23	2.43 (±0.46)	2.902	107.640	−3.41	−3.861	0.12	0.04
24	2.99 (±0.32)	2.874	152.974	−3.87	−3.967	0.04	0.03
25	3.30 (±0.27)	3.339	194.635	−3.94	−4.113	0.04	0.02

methods for compounds **2**, **3**, **8**, **11**, **14**, **22** and **23**. Where there was agreement, compounds could be classified into; those with lower calculated solubility from the interval between 20 and 70 mg/L (compounds **4**, **21** and **24**–**25**), those with calculated solubility from the interval between 0.1 and 3.5 g/L sufficient for fast adsorption, (compounds **1**–**3**, **5**–**7**, **9**–**10**, **15**–**16** and **19**–**20**) and those that had higher calculated solubility, above 7 g/L (compound **13**).

2.4. Polar surface area and 'rule-of-five' properties

Polar surface area (PSA) (the solvent accessible area of the compound which will interact with the solvent in a dipole or hydrogen bond (H-bond) interaction)[37] is, together with lipophilicity and solubility, widely acknowledged as an important factor in transport across membranes. It has been suggested that compounds which are to be passively absorbed should have a maximum PSA of 120 Å^2.[38] In Table 2, compounds **1**–**25** were all predicted using QikProp to have a PSA under this limit. In addition, the total solvent accessible surface area (SASA) and the hydrophilic component of the solvent accessible surface area (FISA) were calculated (QikProp) so as to generate a value for % PSA (FISA/SASA). For the years 1993–2002, the median % PSA for known drugs was 21.2.[39] About half of the compounds listed in Table 2 (Compounds **1**–**4**, **11**, **12**, **14**, **17**–**21** and **23**–**25**) have a % PSA within 5% of this value.

In conjunction with the data in Tables 1 and 2, the predicted properties (QikProp) of compounds **1**–**25** were also checked against Lipinski's 'rule-of-five',[26] a well-known screen for the identification of drug-like properties in a molecule.[40] It should be noted that the fifth rule of the Lipinski 'rule-of-five' specifically excludes natural products and substrates for biological transporters and that another study suggests the lower figure of 350 for molecular weight for lead-like molecules.[33] Table 3 contains the remainder Lipinski parameters (Log *P* is listed in Table 1) for compounds **1**–**25**, of molecular weight (M_W), molecular flexibility (measured by the number of rotatable bonds), and counts of hydrogen bond acceptors and donors in a molecule. All compounds, except for compound **4** (Table 3, Entry 4), have an atomic weight less than 350.

Table 2. Calculated (QikProp) polar surface area (PSA), % PSA (FISA/SASA) for compounds **1**–**25**

Compound	PSA	SASA	FISA	FISA/SASA%
1	87.963	511.780	106.390	20.79
2	87.510	548.965	106.237	19.35
3	88.513	554.534	116.800	21.06
4	85.999	577.081	99.536	17.25
5	116.451	429.445	206.103	47.99
6	116.206	468.377	205.846	43.95
7	116.420	464.942	208.975	44.95
8	97.911	393.276	163.002	41.45
9	89.561	434.838	147.325	33.88
10	90.227	435.176	151.729	34.87
11	74.221	455.208	98.487	21.64
12	100.689	535.666	113.219	21.14
13	101.815	414.903	148.561	35.81
14	85.796	432.045	95.838	22.18
15	101.463	463.489	152.752	32.96
16	99.197	510.272	135.846	26.62
17	101.028	544.834	133.018	24.41
18	97.689	560.008	122.007	21.79
19	88.547	514.410	119.942	23.32
20	102.362	494.445	127.176	25.72
21	92.272	593.109	128.937	21.74
22	93.190	530.077	152.232	28.72
23	94.349	562.797	144.2084	25.62
24	76.152	526.838	128.112	24.32
25	75.592	554.200	117.081	21.13

Hydrogen-bonding capacity has been identified as an important parameter for describing drug permeability.[41,42] An excessive number of H-bond donors (>5) and/or a high number of oxygen and nitrogen atoms ($\geqslant$10) can result in poor absorption. In Table 3, for compounds **1**–**25** the number of H-bond acceptors per compound is <10 (4–8.7) and the number of H-bond donors is $\leqslant$2. The acid or base properties of compounds can greatly affect their bioavailability, as this relates to their charged or neutral state at physiological pH. Brønstead acids have better bioavailability than neutral species and Brønstead bases.[43] Compounds **15**–**25** are monoacids and compounds **5**–**7** are diacids. Reduced molecular flexibility measured by the number of rotatable bonds (optimally below eight),[38] is another important predictor of good oral bioavailability.[41,42] In Table 3, compounds **1**–**25** all have a low number of rotatable bonds <5 as they all possess a high to moderate degree of structural rigidity. As none of compounds **1**–**25** violated the 'rule-of-five' (i.e., Lipinski score = 0, Table 3), and the calculated values for Log *P*, QPPCaco, Log *S*, PSA, %PSA for compounds **1**–**25** were generally within desired limits, all compounds **1**–**25** were screened against GPa.

2.5. GPa assay

GPa activity was measured as described[8] in the direction of glycogen synthesis[44] by measuring the formation of inorganic phosphate from glucose-1-phosphate at 25 °C and against a caffeine standard, and was carefully monitored for signs of insolubility of a compound under assay conditions. The high concentration screening used here is aligned with 'fragment-screening' methods.[45] Data for inhibition of GPa by compounds **1**–**25** are summarized in Table 3. Compounds **1**–**25** showed no correlation between lower solubility (expressed in mM; Table 3) and false activity. Inactive compounds **4, 17** and **22** had predicted solubility concentrations comparable to the predicted solubility concentrations of 0.08–0.24 mM for compounds with an estimated IC_{50} (i.e., **18**, **21** and **23**–**25**). In the GPa assay a low percent component of DMSO is used to solubilise compounds and thus improved compound solubility in the assay should be observed.

Synthetically derived Hyphodermin A, B and D (**13**, **9** and **14**) did not inhibit GP at $\leqslant$1.4–3 mM. This confirms that Hyphodermins A, B and D (**13**, **9** and **14**) do not regulate GPa. However, compound **11**, which is a direct structural analogue of Hyphodermin D (**14**) without the

Table 3. Calculated (QikProp) Lipinski parameters, QPLogS and GPa inhibition assay results for compounds **1–25**

Compound	M_W	Donor HB	Acceptor HB[a]	Acid	Neutral	NROTB	Lipinski score[b]	QPLogS (mM)	IC_{50} (mM)[c]
1	262.3	0	6	No	Yes	4	0	3.563	na[d]
2	290.3	0	6	No	Yes	4	0	0.703	na
3	288.3	0	6	No	Yes	4	0	0.552	na
4	369.2	0	6	No	Yes	4	0	0.102	na
5	234.2	2	6	Yes	No	2	0 (1)	8.712	na
6	262.3	2	6	Yes	No	2	0 (1)	2.326	na
7	260.2	2	6	Yes	No	2	0 (1)	2.620	na
8	216.2	0	6.5	No	Yes	4	0	541.532	na
9	246.3	1	6.7	No	Yes	0	0	11.884	na
10	244.2	1	6.7	No	Yes	0	0	11.742	na
11	258.3	0	6.7	No	Yes	1	0	67.225	na[e]
12	304.3	0	8	No	Yes	4	0	14.007	na
13	260.2	1	8.7	No	Yes	0	0	87.916	na
14	274.3	0	8.7	No	Yes	1	0	2059.022	na
15	248.2	1	6	Yes	No	3	0	3.754	na
16	276.3	1	6	Yes	No	4	0	0.932	na
17	309.3	2	6.5	Yes	No	2	0	0.293	na
18	323.3	2	6.5	Yes	No	4	0	0.244	11.7
19	289.3	1	7	Yes	No	4	0	2.469	na
20	303.3	1	8.7	Yes	No	2	0	8.644	na
21	320.4	1	6	Yes	No	7	0	0.129	1.3
22	309.3	2	5.75	Yes	No	2	0	0.221	na
23	323.3	2	5.75	Yes	No	4	0	0.138	1.2
24	291.3	1	5	Yes	No	2	0	0.108	0.8
25	305.3	1	5	Yes	No	4	0	0.077	1.1

[a] Values are averages taken over a number of configurations.
[b] Number of 'rule-of-three' violations is given in brackets, where found.
[c] IC_{50} value (estimated) for inhibition of GPa (see Section 3).
[d] na, not active against GPa at maximal tested concentration of 0.222–22 mM (see Section 3).
[e] Classified as not active as only ~15% inhibition was observed at 3 mM.

epoxide group, did display marginal inhibition (15%) of GPa at 3 mM.

Furthermore, thioether **18** and cyclic analogues **21**, **23–25** are all new inhibitors of GPa, with estimated IC_{50} values in the range 0.8–11.7 mM. Due to these moderate inhibition values, the K_i values were not determined. As these compounds represent a new structural class of GPa inhibitor, the mode or site of inhibition of GPa is not known for compounds **18**, **21**, and **23–25** and this may influence the maximal inhibitory response observed.

In the context of the assay results, we found the inactivity of compounds **17** and **22**, which display close structural similarity with **18** and **23**, to be unexpected and not readily explained. Although **22** and **23** can eliminate water to form **24** and **25**, respectively, they are stable under the short time length of the assay. Upon comparison of the calculated and screening data (Tables 1–3) for inactive compounds (**1–17**, **19–20** and **22)** to compounds with an estimated IC_{50} (**18**, **21**, and **23–25**), correlations between inhibition and predicted properties such as lipophilicity and solubility, were generally not found. Probably the individual structural features of compounds **18**, **21** and **23–25** are important for activity.

2.6. Molecular modelling

As a first step for future structure–activity relationship studies, we performed conformational searching and energy minimization, applying the GB/SA continuum solvation treatment for water, on compounds **18**, **21**, **23–25** using the Macromodel module within Maestro (Version 8.0.110) modelling package (Schrödinger, Inc.).[46] Superimposition of the lowest energy conformation for each of compounds **18**, **21**, **23–25** (Fig. 3) was achieved using carbons C2a, C3, C8a, and C8b in compounds **23–25** and carbons 1, 2, 8 and 8a in compounds **18** and **21**. From this superimposition it was apparent that the compounds were highly rigid. The low number of rotatable bonds in compounds **21**, **23–24** suggests that these compounds upon binding to a protein such as GPa would change their conformation only slightly. In addition, the superimposition of **18**, **21**, **23–25** (Fig. 3) showed general spatial colocation of the polar carboxylic acid group at C3, and the aromatic side chain groups (i.e., phenyl, benzyl); the latter suggesting a possible van der Waal interaction or induced dipole interaction with GPa. From these results, the structural features of **18**, **21**, **23–25** have been identified as possible moieties for further structure–activity relationship (SAR) studies. The work has identified 2-phenyl and benzyl substituted 2-oxo-hexahydro and 2-oxo-tetrahydrobenzo[*cd*]indole carboxylic acids as a potential new class of molecule for future GPa inhibitor design and/or GPa binding studies.

3. Experimental

Samples of test compounds **1–25** were synthesised as reported elsewhere.[17–19]

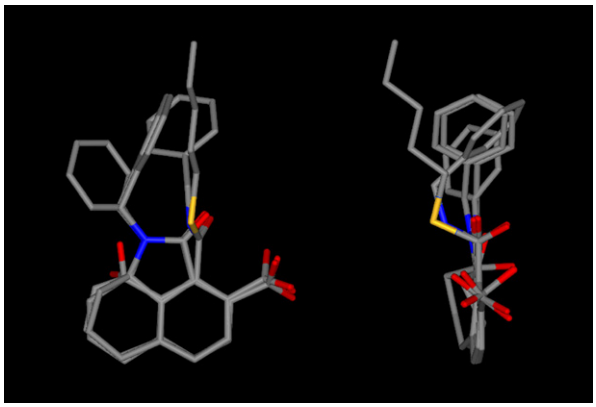

Figure 3. Superimposition of low energy conformers of compounds **18**, **21** and **23–25**, localized about carbons C2a, C3, C8a and C8b in **23–25** and carbons 1, 2, 8 and 8a in **18** and **21**.

3.1. Crystal data for 1-(methoxycarbonyl)-8-oxo-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid (15)

Crystallised as the monohydrate by slow evaporation from chloroform. $C_{13}H_{12}O_5{\cdot}H_2O$ $M = 266.2$, triclinic, space group P-1, $a = 8.1406(11)$, $b = 8.4584(8)$, $c = 9.9940(13)$ Å, $\alpha = 87.520(9)°$, $\beta = 107.341(10)°$, $\gamma = 103.475(9)°$, $U = 638.5(1)$ Å^3, $Z = 2$, $D_c = 1.38$ g cm^{-3}, $\mu = 0.11$ mm^{-1}, Crystal size: $0.50 \times 0.40 \times 0.30$ mm, 2568 reflections collected, 2244 unique ($R_{int} = 0.032$), $R = 0.043$ [1878 reflections with $I > 2\sigma(I)$], $wRF2 = 0.132$ (all data).

Crystallographic data (excluding structure factors) for ester **15** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary Publication No. CCDC 678131. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

3.2. GPa enzyme assay

Rabbit muscle Glycogen Phosphorylase a (from Sigma, 0.475 μg/mL) activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate[47] using a 384-well plate at 22 °C in 45 μl of buffer containing 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM EGTA, 2.5 mM $MgCl_2$, 0.25 mM glucose-1-phosphate, and 1 mg/ml glycogen with a 30 min incubation time. Phosphate was measured at 620 nm, 5 min after the addition of 150 μl of 1 M HCl containing 10 mg/ml ammonium molybdate and 0.38 mg/ml malachite green.[48] Test compounds were added to the assay in 5 μl of 14% DMSO. Compounds were tested against a caffeine standard in 11pt CRC in duplicate on two separate occasions, with Hill slopes between 0.5 and 3.0 and Z' values of ~0.8. Depending on synthetic availability, compounds were also screened at a higher concentration range with maximal concentrations of 0.4 mM (**2**, **7**), 1.4 mM (**3**, **9**, **10**), 1.9 mM (**13**), 3 mM (**11**, **14**), 4.7 mM (**6**, **7**), and 22 mM (**1**, **4**, **8**, **15–25**). Estimated IC_{50} values were determined with curve prediction and with % inhibition observed at 22 mM

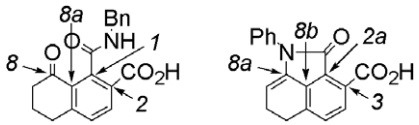

Figure 4. Examples of numbering system used for **18**, **21** and **23–25**.

of; 89% for **18**, 55% for **21**, 65% for **23**, 46% for **24**, 46% for **25**.

3.3. Physicochemical calculations and molecular modelling

Lipinski parameters, polar surface area, lipophilicity, and water based calculations were carried out using QikProp 2.5 (Schrödinger, Inc.).[20] Lipophilicity, and water based calculations were also carried out using the web-based tool ALOGpS 2.1.[21,22] Conformational searching using torsional sampling (MCMM) and energy minimization was carried out on compounds **18**, **21**, **23–25** using the Macromodel module within Maestro (Version 8.0.110) modelling package (Schrödinger, Inc.).[46] For each compound (**18**, **21**, **23–25**) multiple random conformations were generated. The number of random conformations for **18**, **21**, **23–25** varied, depending on the number of rotatable bonds, and was 8000, 11,000, 6000, 4000 and 5000, respectively. Each random conformations was minimized using the Truncated Newton Conjugate Gradient method (TNCG) with the OPLS_2005 force field. The GB/SA continuum solvation treatment for water was used in all minimizations. The minimization convergence criteria was considered to be complete when the gradient was less than 0.05. The superimposition of lowest energy conformations for compounds **18**, **21** and **23–25** was achieved using carbons C2a, C3, C8a and C8b in compounds **23–25** and carbons 1, 2, 8 and 8a in compounds **18** and **21** (see Fig. 4).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.047.

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