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Norcantharidin Analogues: Synthesis, Anticancer Activity and Protein Phosphatase 1 and 2A Inhibition

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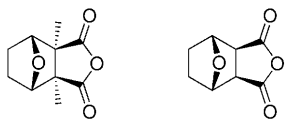
*Cantharidin (**1**) and its derivatives are of significant interest as serine/threonine protein phosphatase 1 and 2A inhibitors. Additionally, compounds of this type have displayed growth inhibition of various tumour cell lines. To further explore both of these inhibition pathways, a number of amide–acid norcantharidin analogues (**15**–**26**) were prepared. Compounds **23** and **24**, containing two carboxylic acid residues, showed good PP1 and PP2A activity, with IC_{50} values of ~15 and ~3 μM, respectively. Substituted aromatic amide analogues **45**, **48**, **49**, **52**, **53**, and **54** also displayed good PP1 and PP2A inhibition, with IC_{50} values in the range of 15–10 μM (PP1) and 11–5 μM (PP2A). However, bulky* ortho *substituents on the aromatic ring caused the aromatic ring to be skewed from the NCO planarity, leading to a decrease in PP1 and PP2A inhibition. A number of analogues, **20**, **22**, **25** and **46**, showed excellent tumour growth inhibition, with **46** in particular being more potent than the lead, norcantharidin **2**.*

Introduction

Mylabris, the dried body of the Chinese blister beetle (*M. phalerata* and *M. cichorii*) has been used for the treatment of a range of conditions, from warts to piles, for over 200 years.[1–3] More recent examination of mylabris has highlighted cantharidin (**1**, (3a*R*,4*S*,7*R*,7a*S*)-hexahydro-3a,7a-dimethyl-4,7-epoxyisobenzofuran-1,3-dione) as the active component. Cantharidin (**1**) is found in the Meloidae family of Coleoptera (beetles) where it is used as a potent defensive agent. This compound is common to ~1500 species of beetle from India, China and North America, and derives its name from the Spanish Fly (*Cantharris vesicatoria*) found in the Mediterranean area. The first reported association of mylabris with anticancer activity dates back to 1264.[4] Since this initial reference, use of the natural product **1** in western medicine has been limited, conceivably due to its toxicity, and in particular nephrotoxicity.[5–15] More recently, cantharidin (**1**) has been shown to inhibit cell growth in various tumour cell lines including cervical, tongue, ginival, mucoepidermoid, adenocystic, neuronal, bone, leukaemia, ovarian, and colon cancer,with GI_{50} values of 1.3–15 μM.[1–3,9–11,16–20]

Cantharidin (**1**) and its analogues, such as norcantharidin (**2**), are known inhibitors of serine/threonine protein phosphatase.



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These enzymes, responsible for the dephosphorylaton of proteins, have been implicated in the etiology of various disease processes and are considered a novel target for cancer drug therapy.[21–24] Of the serine/threonine protein phosphatases (PP1, PP2A, PP2B, PP2C and others), PP1 and PP2A are the most scrutinised pharmacologically due to their predominance in cells. Genetic mutation studies in yeast and drosophila identify PP1 and PP2A as potential targets for the treatment of cancer. Yeast PP1 mutants are unable to complete the anaphase or instigate chromosome segregation, while the same mutation in drosophila leads to defective spindle organisation and a delay in mitosis progression among other cell cycle abnormalities. Yeast mutations lacking one of the PP2A subunits show a defect in cell septation and separation whereas the same defect in drosophila leads to abnormal anaphase resolution.[30] Inhibition of PP1 and PP2A by cantharidin, and similar by other natural products such as okadaic acid (**3**), tautomycin (**4**) and fostriecin (**5**), has attracted a great deal of attention.[1–3,25–30]

X-ray crystallography and homology modelling studies indicate that the catalytic domain of PP1 and PP2A are similar. Both contain a N-terminal subdomain, a metal atom embedded in the phosphoesterase motif, and an important hydrogen bonded Asp–His moiety.[31] Additionally, site directed mutagenesis studies of the PP1 active site with various natural toxins highlighted the various roles of the active site residues.[30] Structure–activity relationship (SAR) studies on the more potent known PP inhibitors, such as okadaic acid (**3**), tautomycin (**4**) and fostriecin (**5**), have been limited due to their

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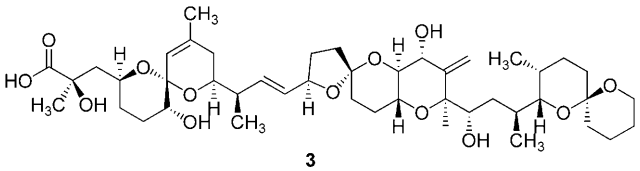

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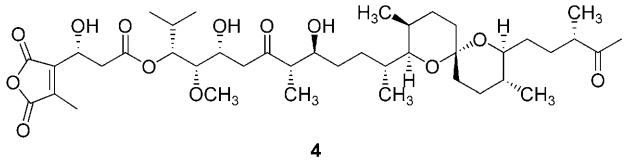

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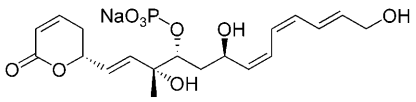

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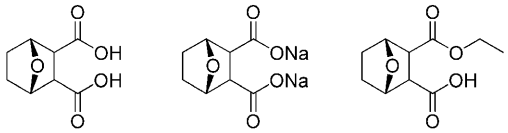
structural complexity and long total syntheses.[25–30] In contrast, cantharidin (**1**) is a relatively simple molecule and with a wide range of potential synthetic derivatives.

Norcantharidin (**2**), the demethylated analogue of cantharidin (**1**), is more synthetically accessable and exhibits similar anticancer activity without the associated nephrotoxicity.[4] Several groups, including our own, have reported SAR studies of compounds **1** and **2** in order to ascertain the key inhibitory features of these molecules.[16–20,32–37] Evaluation of norcantharidin derivatives as PP inhibitors showed that analogues containing a C5=C6 lost some potency, although a few compounds exhibited some selectivity between PP1 and PP2A.[33,35] The C7–O bridgehead is involved in a key hydrogen bonding interaction with the protein phosphatase, consequently, modifications to this moiety are detrimental to the PP inhibition. Tatlock. et al. showed that C5-substituted analogues exhibited enhanced binding to PP2B, but weaker inhibition of PP1 and PP2A.[34] Likewise, C4 or C7 substitution generally led to poorer PP1 and PP2A inhibition, while some PP2B binding was observed. Some cantharidin analogues containing a C7a ring junction side chain, instead of a methyl group, have PP2A inhibitory activity similar to that of the natural product **1**.[36]

Of the norcantharidin carbocyclic skeleton, the anhydride moiety has been most extensively studied. This functionality lends itself to simple nucleophilic substitution reactions from which a a library of analogues could be generated. Ring opened derivatives diacid **6**, bis-sodium salt **7** and acid ester **8** showed only slight differences in activity against PP2A compared with the anhydride system of compounds **1** and **2**.[1–3] Importantly, while the cyclic anhydride is not essential, an acid moiety, either inherant in the molecule or arising through the partial hydrolysis of the anhydride, is essential for PP1 and PP2A inhibition.[33] Replacing the anhydride with a cyclic imide containing an *N*-substituted group (i.e. amino acids) has been explored by our group and others.[17,32] Subsequent studies exploring various amines containing a carboxylic acid (ring opened analogues), have provided various PP1 and PP2A inhibitors, which displayed growth inhibition in various tumour cell lines, but none as potent as compound **1**.[20]



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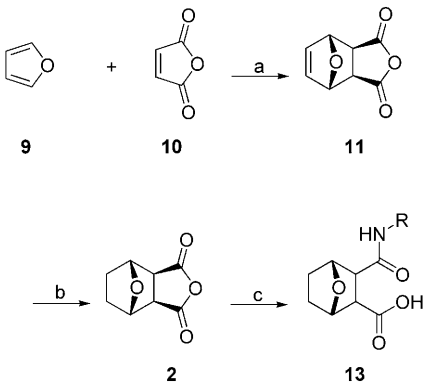
The relatively facile preparation of large quantities of norcantharidin **2** allowed us to generate a library of simple ring opened analogues and evaluate their potential as anticancer agents; our efforts in this area are reported herein.

Results and Discussion

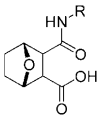
5,6-Dehydrocantharidin **11** was prepared on a large scale through the cycloaddition of the readily available furan **9** and maleic anhydride **10**. Subsequent hydrogenation of *exo*-**11** was carried out following a modified procedure originally described by Eggelte et al., providing the norcantharidin (**2**) in excellent yields.[38] Treatment of the key intermediate **2** with a variety of amines at room temperature in THF rapidly generated the corresponding series of acid amides with the general structure **13** (Scheme 1).[20]

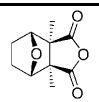
The acid–amide analogues **14**–**21** were tested for PP1 and PP2A inhibition at 100 μM, and those analogues showing >50% inhibition were then subjected to a full dose response analysis (Table 1). All acid–amide analogues (**14**–**18**) tested maintained a moderate level of PP2A selectivity (~2- to 3-fold) comparable to that of lead compound **2**. Of these, the most potent PP inhibitor was derivative **18** (PP1 $IC_{50}=25\pm7$ and PP2A $IC_{50}=8.6\pm1.5$ μM), while analogues **14**–**17** were modest to weak inhibitors of PP1 ($IC_{50}=70\pm6$–25 ± 7 μM) and modest to good inhibitors of PP2A ($IC_{50}=25\pm5$–8.6 ± 1.5 μM). Analogues **19** and **21** were insoluble in the assay media at the stock concentration, presumably due to the long hydrophobic chain (C_{12}, C_{18}). While analogue **20** (C_{14}) was soluble in the assay media, it failed to inhibit PP1 activity and only had a minimal inhibitory effect PP2A (37% reduction at 100 μM), indicating that while potency was compromised, PP2A selectivity was maintained. Collectively, extension of the alkyl chain did



Scheme 1. *Reagents and conditions*: a) RT, 48 h, Et_2O; b) 4 atm H_2, 10% Pd-C, acetone, 3 days; c) RNH_2, THF, RT 16 h.

Table 1. Inhibition of Protein Phosphatases 1 and 2A and growth inhibition of compounds **1**, **2** and **14–22**.[a,b]



Compound		Enzyme Inhibition IC_{50} [μM] or [%]			Cell Line Growth Inhibition GI_{50} [μM] or [%]								
		PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
1		6.0 ± 0.7	0.9 ± 0.08	~6.7	3.2 ± 0.1	4.5 ± 0.3	7.5 ± 0.4	4.4 ± 0.3	3.3 ± 0.2	2.9 ± 0.2	2.1 ± 0.3	3.7 ± 0.6	1.7 ± 0.1
2		7.7 ± 0.6	2.7 ± 0.05	~2.9	57 ± 5	44 ± 6	68 ± 4	38 ± 1	45 ± 3	31 ± 1	28 ± 3	43 ± 6	23 ± 3
14		46 ± 4	18 ± 1	~2.6	*(52±6)*	*(38±1)*	*(16±3)*	*(17±2)*	*(24±4)*	*(57±1)*	*(28±9)*	*(34±8)*	*(63±6)*
15		70 ± 6	24 ± 5	~2.9	94 ± 3	89 ± 1	*(2±9)*	*(43±4)*	*(77±5)*	86 ± 8	*(66±7)*	75 ± 5	62 ± 1
16		35 ± 5	13 ± 0	~2.7	*(66±1)*	*(97±3)*	*(80±3)*	*(71±4)*	*(90±7)*	*(91±1)*	*(81±3)*	*(100)*	*(83±2)*
17		56 ± 9	25 ± 5	~2.2	49 ± 3	36 ± 1	68 ± 7	53 ± 4	36 ± 2	36 ± 5	53 ± 3	28 ± 5	29 ± 3
18		25 ± 7	8.6 ± 1.5	~2.9	*(58±6)*	*(58±1)*	*(21±15)*	*(55±4)*	*(45±6)*	*(71±4)*	*(57±7)*	*(37±4)*	*(74±3)*
19		_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
20		*(0)*	*(37±4)*	–	10 ± 2[d]	11 ± 1[d]	13 ± 1[d]	13 ± 0[d]	14 ± 1[d]	6.8 ± 0.3[d]	14 ± 1[d]	8.7 ± 0.9[d]	9.5 ± 1.3[d]
21		_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
22		40 ± 2	10 ± 4	~4	32 ± 2	16 ± 2	32 ± 9	12 ± 0	95 ± 4	46 ± 3	35 ± 2	16 ± 0	36 ± 3

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50}; μM) or cell growth by 50% (GI_{50}; μM) relative to an untreated control. [b] Values in (*italics*) are percentage enzyme or growth inhibition at 100 μM drug concentration. [c] Insoluble in testing medium. [d] Stock solution prepared at 10 mM to facilitate screening.

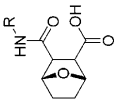
not affect selectivity, however; it did influence potency with analogue **18** showing the optimal configuration. Whilst all analogues were considerably weaker PP inhibitors than norcantharidin **2** (PP1, $IC_{50} = 7.7 \pm 0.6$; PP2A, $IC_{50} = 2.7 \pm 0.05$ μM), the introduction of a long alkyl chain is expected to improve cellular uptake of these molecules and enhance cytotoxicity. As such, the aims of this work were: 1) the evaluation of structural modifications upon PP inhibition, and 2) investigation into the effects of these modifications upon cellular uptake and cytotoxicity.

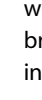
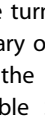
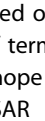
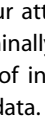
Having determined the protein phosphatase inhibition of the analogues **1**, **2**, and **14**–**22** (Table 1), the cytotoxicity of these analogues was evaluated using a MTT cytotoxicity assay.[19] Compounds **1**, **2**, and **14**–**22** were screened against a panel of human cancer cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BEC-2 (neuroblastoma), and SJ-G2 (glioblastoma). All analogues were screened at a preliminary dose of 100 μM. Derivatives showing consistently high growth inhibition, or a combination of modest growth and PP inhibition, were subjected to full growth inhibition (GI_{50}) determinations (Table 1).

The data displayed in Table 1 shows that analogues **14**–**18** exhibited low levels of cytotoxicity in the cancer cell lines tested, while analogue **20** induced good levels of cytotoxicity (H460, $GI_{50} = 14 \pm 1$; A431, $GI_{50} = 6.8 \pm 0.3$ μM). Interestingly, there is a clear trend of increased cytotoxicity with progressive extension of the alkyl chain (C_3-C_8, C_{18}) for analogues **14**–**17** and **21**, respectively. This observation is encouraging and supports our hypothesis that biological activity is enhanced by extension of the alkyl chain. The only exception to this observation is compound **18** (C_{10}) which showed a biological effect similar to that of **14** (C_3) and **15** (C_4), suggesting that the mode of action of **18** in this biological system is different to that of the other acid amide analogues. In this regard, compound **18** was the only analogue showing potent PP inhibition (Table 1), with solubility issues precluding IC_{50} determination for analogues **19** and **21**. Introduction of a terminal alkene moiety (compound **22**) had only a marginal effect on PP activity relative to the analogous saturated compound **14**, but did result in a significant improvement in the observed cytotoxicity. We note that derivative **22** displayed good to poor cytotoxicity against all cell lines tested, with GI_{50} values ranging from 12 ± 0 (A2780) to 95 ± 4 μM (H460). The discrepancy between PP inhibition and observed cytotoxicity could be due to increase cell penetration; however, this was not confirmed. Moreover, each cell line possesses different cellular machinery associated with cell death which could also give rise to the observed discrepancies.

In our quest to improve PP inhibition and biological activity we turned our attention to the synthesis of a small-targeted library of terminally functionalised amide analogues (Scheme 1), in the hope of increasing solubility, allowing us to obtain valuable SAR data. Specifically, these terminally functionalised amide analogues were designed to improve hydrogen bonding between the substrate and the PP active site. Table 2 summarises the data, which shows that the introduction of hydro-

Table 2. Inhibition of Protein Phosphatases 1 and 2A by acid amide analogues and growth Inhibition of compounds **23**–**28**.[a,b]



Compound	R	Enzyme Inhibition IC_{50} [μM] or [%]			Cell Line Growth Inhibition GI_{50} [μM] or [%]							
		PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
23		15 ± 2	3.3 ± 0.2	~4.6	50 ± 0	55 ± 2	65 ± 3	60 ± 0	60 ± 0	42 ± 4	52 ± 1	65 ± 4	32 ± 6
24		14 ± 1	3.5 ± 0.4	~4	53 ± 2	57 ± 2	61 ± 4	64 ± 1	62 ± 1	40 ± 1	56 ± 2	60 ± 6	30 ± 5
25		23 ± 1.0	13 ± 1.0	~1.8	32 ± 6	23 ± 3	47 ± 5	57 ± 1	29 ± 3	29 ± 3	31 ± 3	15 ± 1	31 ± 3
26		> 100	70	> 1.4	*(54±5)*	*(77±2)*	*(59±6)*	*(60±10)*	*(70±7)*	*(74±4)*	*(51±5)*	*(84±8)*	*(78±4)*
27		16 ± 10	13 ± 12	~1.2	*(6.2±5.1)*	*(6.0±2.2)*	*(11±4)*	*(16±3)*	*(2.2±0.6)*	*(12±6)*	*(0)*	*(13±2)*	*(7.4±4.6)*
28		*(35±6)*	*(56±9)*	–	*(21±5)*	*(33±5)*	*(17±2)*	*(20±2)*	*(25±5)*	*(32±3)*	*(14±3)*	*(23±2)*	*(27±4)*

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50} μM) or cell growth by 50% (GI_{50} μM) relative to an untreated control. [b] Values in *(italics)* are percentage enzyme or growth inhibition at 100 μM drug concentration.

philic functionality does enhance PP inhibition. The addition of a second carboxylate functionality (compounds **23** and **24**) improved both the potency against PP1 and PP2A, and selectivity towards PP2A, when compared to their non-functionalised counterparts **15** and **16**. Continued chain elongation (analogue **25**) was detrimental to PP2A selectivity. As with previous investigations, the presence of a ring-opened motif containing a free carboxylate group results in a demonstrable increase in potency (Table 2) compared to their less polar analogues (Table 1).[17] The second carboxylate is clearly beneficial (**24** vs. **26**), although a similar improvement can be seen with the introduction of an amine linker, as with analogue **27** (PP1, $IC_{50} = 16 \pm 10$; PP2A, $IC_{50} = 13 \pm 12$ μM). Further investigations on more functionalised derivatives similar to analogue **24** are currently underway.

The alkyl chain length was examined in an attempt to optimise inhibitor interactions with the hydrophobic cleft of the protein (Figure 1).[40] Analogues **23** and **24** are potent PP2A inhibitors, with IC_{50} values of 3.3 ± 0.2 and 3.5 ± 0.4 μM, respectively; chain elongation did not effect potency in these compounds, however, a chain elongation to C_8 (compound **25**) resulted in a fourfold decrease in PP2A inhibition, and a 1.5-fold decrease in PP1 inhibition. Generally, this series of compounds showed improved selectivity for PP2A over PP1 inhibition, up to ~4.6-fold (analogue **23**), together with increased enzyme inhibition. Interestingly, incorporation of a terminal imidazole moiety had an adverse effect on PP inhibition (analogue **28**, Table 2), this may be the result of increased hydrogen bond acceptors or the aromatic nature of the ring as similarly sized compounds **22** and **23** maintained good levels of PP inhibition. Given that PP inhibition was measured using purified enzymes, the adverse effects noted cannot be a result of poor cellular uptake.

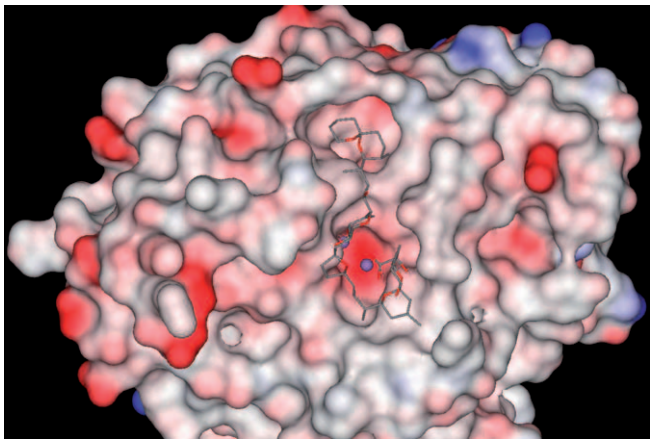
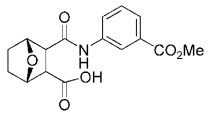
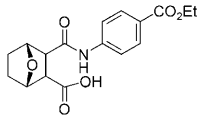


Figure 1. The PP2A–Okadaic acid co-crystal structure.[31]

These analogues were subsequent screened for cytotoxicity against a number of tumour cell lines using an MTT assay.[16] Interestingly, analogues **23**–**25** showed an improvement in cytotoxicity (Table 2) compared with the corresponding alkyl analogues **15**–**17** (Table 1), this indicates that the inclusion of a second carboxylate not only improved PP inhibition, but also increased cytotoxicity. A pattern of improved growth inhibition through the extension of the amide side chain **23**–**25** was also apparent. No significant activity was displayed for **26**–**28**, which in the case of **27** is most likely due to an inability to cross the cell membrane, presumably as a result of the amino moiety.

The synthetic availability that previously hindered the systematic examination of the structural requirements of PP inhibitors based on cantharidin (**1**) has been largely overcome by our simple route to the key intermediate norcantharidin (**2**) (Scheme 1). The study of simple alkyl and terminally functionalised alkyl amide derivatives generated potent PP1 and PP2A inhibitors, some of which are potent cytotoxics. In an effort to further evaluate more functionalised amide derivatives, we returned to our previously reported anilino-substituted analogues **29** and **30** (Table 3), which are modest inhibitors of both PP1 and PP2A displaying ~2 fold PP2A selectivity, however, they are poorly cytotoxic.[20] The poor correlation between PP inhibition and cytotoxicity arises through various factors such as cell permeability, compound transport (active), drug metabolism, and efflux mechanisms (via P-glycoproteins) are all additional complications that may mask any such correlation.

Table 3. Inhibition of Protein Phosphatases 1 and 2A and growth inhibition of anilino analogues **29** and **30**.[a]

Compound		Enzyme Inhibition IC_{50} μM		Growth Inhibition GI_{50} μM				
		PP1	PP2A	A2780 Ovarian	G401 Kidney	HT29 Colorectal	H460 Lung	L1210 Murine
29		16 ± 2	7.7 ± 1.3	90 ± 0.1	> 100	> 100	> 100	> 100
30		29 ± 11	9.2 ± 2.9	80 ± 5.8	> 100	> 100	> 100	> 100

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50} μM) or cell growth by 50% (GI_{50} μM) relative to an untreated control.

The commercial availability of substituted anilines provided scope for the further evaluation of aromatic amide substituents. Small libraries with variations in the aromatic substituents were developed. Firstly, a series of simple mono-substituted aromatic amides were screened for PP1 and PP2A inhibition (Table 4). These compounds exhibited good to modest inhibition of these enzymes. Derivative **31** was twice as potent against PP activity (PP1, $IC_{50}=24\pm2.8$; PP2A, $IC_{50}=7.7\pm0.8$ μM) compared with the most potent linear analogue **24**. Halogen substituted analogues **32**–**36** exhibited a decrease in PP inhibition ranging from 1.25- to threefold for PP1; and 2- to threefold for PP2A. These effects were most pronounced for the *ortho*-Cl derivative **32**, which showed a threefold decrease in PP1 and a twofold decrease in PP2A inhibition relative to the unsubstituted aromatic compound **31**. Greater potencies were observed for *meta*- or *para*-substituted halogenated analogues, but all were less active than the parent compound **31** suggesting that, whilst the *meta* and *para* position have a higher tolerance for electron-withdrawing groups and/or bulky substituents, halogen substituents do not impart favourable PP inhibition characteristics. Introduction of a *para*-NO_2 (compound **37**) reduced potency twofold relative to the para-iodo derivative **36**; strong electron withdrawing groups were not tolerated at this position. Introduction of electron donating groups at *ortho* position had a similar detrimental effect on PP inhibition, for example *ortho*-OH substitution in compound **38** rendered the compound inactive, perhaps due to an intramolecular hydrogen bond between the phenol and carboxylate carbonyl group leading to perturbation of the aromatic ring planarity. A minimum of one free carboxylate is required for activity of this class of compounds against PP1 and PP2A.[33] Both the *para*-OH (**39**) and *para*-COOH (**40**) substituted compounds were equipotent to the unsubstituted analogue **31**, this substantiates the aforementioned intramolecular hydrogen bonding hypothesis associated with the lack of activity of compound **38**. The *para*-OCH_3 substituted analogue **41**, which displays improved nonspecific PP inhibitory activity contrary to the normal PP2A selectivity of norcantharidin analogues, gives further evidence for the tolerance of steric bulk at the *para* position. Bioisosteric manipulation of analogue **41** ($OCH_3\rightarrow SCH_3$) gave analogue **42**, which displays modest PP1 selectivity and good overall PP inhibition (PP1, $IC_{50}=10\pm4.1$; PP2A, $IC_{50}=14\pm1$ μM; PP1/PP2A = 0.71). A similar effect was noted for the *para*-OC_5H_{11} derivative **44** with PP1/PP2A = 0.73, however, extension of the alkyl chain to C_8 (compound **45**) reverted selectivity to PP2A (~3 fold), and induced a significant decrease in PP inhibition. The position of the substituent on the aromatic ring appeared pivotal to maintaining good PP inhibition, a simple relocation of the -SCH_3 moiety from the *para* (compound **42**) to the *ortho* (compound **43**) position rendering the compound inactive. Thus suggesting either a low tolerance for steric bulk in the receptor site at this position, or that the aromatic ring is distorted relative to the amide bond leading to an adverse affect on activity.

Analogues possessing *para* substituents (e.g. compounds **41** and **42**) displayed good PP inhibition. Simple modifications allowed us to remove the *para* functional group from the bicyclo[2.2.1]heptane unit via chain extension. Nine such derivatives (compounds **46**–**54**) were generated from commercially available starting materials, and were examined for PP inhibition and cytotoxicity (Table 4). Disappointingly we did not observe any real increase in either PP inhibition or cytotoxicity. As noted earlier *ortho* substitution removed activity (compound **46**), and substituents in the *meta* and *para* positions were well tolerated and retained PP activity but failed to elicit any improved activity. The morphilino analogue (**54**) exhibited both poor PP inhibition and cytotoxicity. However, the other eight derivatives (**46**–**53**) in this series maintained good PP inhibition, but all displayed very low levels of cytotoxicity. We believe that this is a function of poor membrane penetration, rather than poor PP inhibition.

Of all the analogues described in Table 4, the only substituted aniline derivatives to exhibit excellent to modest levels of cytotoxicity and PP inhibition were derivatives **44** and **45**, in particular the C_8 ether *meta*-substituted analogue **45** displayed good PP2A selectivity. We believe that the C_8 chain improves cell permeability allowing rapid cellular uptake, and that PP2A specificity imparts a degree of cytotoxicity. This is in keeping with our previous observation that long alkyl substituted analogues returned modest to good levels of cytotoxicity, for example, compound **20** (Table 1).

Compound **38** highlighted the importance of the intramolecular interactions within these simple analogues with the formation of an internal hydrogen bond from the *ortho*-OH to the free carboxylate rendering it inactive. In an effort to further substantiate and explore the possible ramifications of this observation, we synthesised a series of alkyl substituted derivatives **55**–**65** that would allow perturbation of the aromatic moiety relative to the amine (HN–CO), and tested them for their PP inhibition and cytotoxicity (Table 5).

Introduction of a single *ortho* alkyl substituent adversely affected both PP inhibition and cytotoxicity. The ethyl substituted compound **55** is approximately threefold less active as a PP inhibitor than the unsubstituted aromatic derivative **31**, whereas the *tert*-butyl analogue **56** is inactive. While a *para*-substituted *tert*-butyl group (analogue **57**) restores PP inhibitory activity. These results indicate that any substitution pattern that forces the aromatic ring out of plane of the amide bond is detrimental to PP inhibition and similarly to cytotoxicity (Table 5, compounds **55**, **56**, **58**, **60**, **61** and **64**). Notably, the most potent PP inhibitor **62** (PP1, $IC_{50}=8.2\pm2.5$; PP2A, $IC_{50}=8.2\pm0.7$ μM) in this series was also the most cytotoxic with GI_{50} values of 28–66 μM.

This data strongly suggests that *ortho* substituents are of limited utility. Increasing complexity (and steric bulk) skews the aromatic ring from its optimal alignment with the amide; indeed simple modelling analysis confirms this (data not shown). Logically, placing the aromatic ring more remote to the amide functionality should reintroduce some level of PP inhibition and cytotoxicity. This was confirmed by the synthesis and evaluation of a series of analogues with a variety of substituents commencing with methylene spacer units (Table 6).

Generally, these analogues were good to modest PP2A inhibitors, with a few notable exceptions. Analogue **70** was a

Table 4. Inhibition of Protein Phosphatases 1 and 2A and growth inhibition of compounds **31–54**.[a, b]

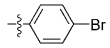
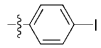
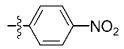
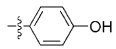
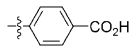
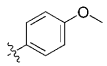
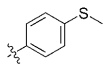
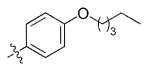
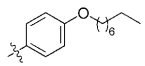
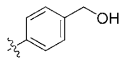
	Compound	Enzyme Inhibition IC_{50} [μM] or [%]			Cell Line Growth Inhibition GI_{50} [μM] or [%]								
		PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
31		24±2.8	7.7±0.8	3.12	*(38±1)*	*(29±3)*	*(38±3)*	*(17±2)*	*(15±3)*	*(33±1)*	*(12±3)*	*(21±3)*	*(36±5)*
32		75±8.7	20±1.3	3.75	*(47±3)*	*(13±2)*	*(17±3)*	*(9±2)*	*(5±3)*	*(15±1)*	*(5±2.2)*	*(10±3)*	*(54±5)*
33		31±4.3	14±0.9	2.21	*(40±1)*	*(27±1)*	*(25±2)*	*(10±3)*	*(12±5)*	*(37±6)*	*(11±5)*	*(54±12)*	*(41±3)*
34		35±7.5	14±2.2	2.50	*(44±1)*	*(36±2)*	*(27±5)*	*(23±1)*	*(17±2)*	*(38±6)*	*(2±6)*	*(40±8)*	*(41±4)*
35		44±1.2	18±0.7	2.44	*(44±6)*	*(31±1)*	*(27±1)*	*(28±15)*	*(10±3)*	*(34±4)*	*(8±4)*	*(45±2)*	*(47±1)*
36		33±6.5	14±1.2	2.36	*(63±2)*	*(52±1)*	*(52±5)*	*(37±0.4)*	*(27±6)*	*(48±6)*	*(8±4)*	*(60±9)*	*(56±2)*
37		74±13	23±1.5	3.22	*(48±3)*	*(15±3)*	*(27±4)*	*(19±3)*	*(<0)*	*(21±1)*	*(6±5)*	*(23±3)*	*(52±4)*
38		*(9.8±11)*	*(33±19)*	–	*(26±3)*	*(12±3)*	*(38±7)*	*(30±5)*	*(12±5)*	*(28±3)*	*(2±3)*	*(16±8)*	*(28±10)*
39		28±3.2	7.7±1.1	3.94	*(23±6)*	*(10±4)*	*(19±6)*	*(7±5)*	*(1±2)*	*(13±3)*	*(<0)*	*(5±3)*	*(22±5)*
40		28±3.2	7.2±0.4	3.89	*(39±2)*	*(28±2)*	*(23±2)*	*(11±6)*	*(14±1)*	*(33±4)*	*(16±3)*	*(50±8)*	*(46±1)*
41		16±0.5	16±0.7	1	*(39±2)*	*(33±1)*	*(20±1)*	*(19±2)*	*(16±1)*	*(37±3)*	*(19±2)*	*(35±4)*	*(35±1)*
42		10±4	14±1.0	0.71	*(59±4)*	*(70±4)*	*(55±4)*	*(35±1)*	*(52±3)*	*(53±4)*	*(33±7)*	*(60±1)*	*(46±2)*
43		*(58±4)*	*(76±5)*	–	*(31±8)*	*(23±2)*	*(60±10)*	*(23±4)*	*(10±3)*	*(11±4)*	*(<0)*	*(24±2)*	*(13±2)*
44		11±4.9	15±1.5	0.73	41±4	54±2	45±5	84±12	90±10	58±4	80±7	49±7	72±4
45		60±15.3	20±4.2	3.0	3.5±1.2	2.3±1.4	1.0	9.2±1.3	31±0.0	10±1.2	40±0.6	6.4±1.7	26±0.7
46		*(30±6)*	*(84±3.2)*	–	*(18±9)*	*(9±2)*	*(3±1)*	*(11±1)*	*(8±4)*	*(16±1)*	*(4±2)*	*(10±3)*	*(10±2)*
47		15±1.7	7.2±2.0	2.1	*(36±4)*	*(48±4)*	*(26±1)*	*(18±8)*	*(31±3)*	*(36±3)*	*(35±6)*	*(12±6)*	*(41±1)*
48		15±0.3	7.0±1.5	2.1	*(41±2)*	*(52±2)*	*(24±2)*	*(22±3)*	*(33±4)*	*(41±4)*	*(38±4)*	*(34±4)*	*(41±2)*

Table 4. (Continued)

Compound	Enzyme Inhibition IC_{50} [μM] or [%]				Cell Line Growth Inhibition GI_{50} [μM] or [%]							
	PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
49	23±0.0	11±1.0	2.1	*(40±4)*	*(52±4)*	*(32±5)*	*(61±4)*	*(41±6)*	*(32±2)*	*(35±3)*	*(40±4)*	*(39±3)*
50	20±5.0	16±1.0	1.3	*(29±2)*	*(28±6)*	*(16±4)*	*(25±1)*	*(12±5)*	*(25±1)*	*(18±2)*	*(18±1)*	*(25±3)*
51	15±3.0	5.7±1.2	2.8	*(24±3)*	*(43±4)*	*(24±8)*	*(16±5)*	*(27±3)*	*(39±4)*	*(28±2)*	*(25±1)*	*(30±5)*
52	10±0.6	4.6±0.7	2.2	*(48±3)*	*(61±3)*	*(24±6)*	*(12±2)*	*(42±3)*	*(48±1)*	*(44±2)*	*(38±2)*	*(48±3)*
53	12±3.2	5.4±0.8	2.2	*(30±2)*	*(46±3)*	*(20±1)*	*(20±3)*	*(29±2)*	*(34±2)*	*(32±2)*	*(14±3)*	*(34±3)*
54	*(82±2)*	*(89±3)*	–	*(23±2)*	*(29±1)*	*(21±3)*	*(18±4)*	*(19±3)*	*(21±4)*	*(32±2)*	*(13±4)*	*(21±2)*

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50}; μM) or cell growth by 50% (GI_{50}; μM) relative to an untreated control. [b] Values in (*italics*) are percentage enzyme or growth inhibition at 100 μM drug concentration.

weak inhibitor, presumably due to the effect of the electron withdrawing chloro substituent, as the corresponding electron donating methoxy derivative **68** was significantly more potent against both PP1 and PP2A. Alkyl spacers shorter than 4 methylene units had a limited effect on PP inhibition, however, compound **74** (C_4) showed considerably reduced enzyme inhibition. This is somewhat surprising given that these analogues are thought to bind in the hydrophobic groove of the protein, and that the microcystins, known PP inhibitors, have a similar aromatic terminated hydrophobic tail, which is longer than the butyl tail associated with **74**.[1–3,26,39] We expected the longer alkyl chains to improve inhibition, with the limiting factor being solubility, as observed with the simple alkyl substituted analogues in Tables 1 and 2. We also hypothesised that active PP inhibitors with the ability to cross cell membranes would display good levels of cytotoxicity, i.e. we believed that inhibition of PP and cytotoxicity were linked. In the majority of cases hypothesis was substantiated, and in those instances were analogues display good PP inhibition but poor cytotoxicity, polar moieties were present in the structure that may have adversely affected their ability to reach the target protein within the cell.

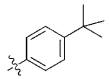
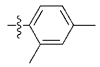
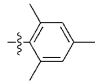
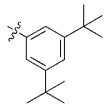
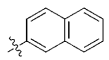
Conclusions

We have successfully developed a number of new norcantharidin analogues with good to poor levels of PP inhibition. In a number of instances an excellent correlation between PP inhibition and cytotoxicity was observed, although there is a delicate balance between the requirements for cellular uptake and solubility in the assay media that complicates the confirmation of our hypothesis of PP inhibitors having broad spectrum anticancer activity. A large amount of SAR data was compiled through a simple divergent based approach from a readily accessible key intermediate **2**. Enzyme inhibition studies highlighted several key structural features needed for inhibition including an amide-α-carboxylic acid motif as an alternative to a cyclic anhydride. Additionally, an improvement in potency was observed when an additional carboxylic acid group was added to the amide R group, as in compounds **23** and **24**. All of the synthesised compounds were also evaluated for their growth inhibition against various tumour cell lines, in particular compounds **20** (GI_{50} = ~11 μM), **22** (GI_{50} = ~36 μM), **25** (GI_{50} = ~33 μM) and **45** (GI_{50} = ~14 μM) showed improved cytotoxicity over compound **2** (GI_{50} = ~42 μM). The most significant improvements were noted for the *para*-substituted derivative **45**, which displayed PP and growth inhibition values greater or equal to those observed for compound **2**, and arguably equal to those observed for compound **1**. This study provides further evidence in support of the development of PP inhibitors as antitumour agents.

Experimental Section

Materials: All reagents were of commercial quality and were used as received (Sigma–Aldrich). Solvents were dried and purified using standard techniques. Reactions were monitored by TLC, using

Table 5. Inhibition of Protein Phosphatases 1 and 2A by acid amide analogues and growth inhibition of compounds **55**–**65**.[a,b]

Compound		Enzyme Inhibition IC_{50} [μM] or [%]			Cell Line Growth Inhibition GI_{50} [μM] or [%]								
		PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
55		51±5.2	24±3.3	2.1	*(15±2)*	*(20±2)*	*(13±5)*	*(3±3)*	*(4±3)*	*(13±2)*	*(<0)*	*(13±3.4)*	*(15±3)*
56		*(16±5)*	*(9±0.3)*	–	*(42±5)*	*(23±2)*	*(71±1)*	*(25±3)*	*(11±2)*	*(12±3)*	*(<0)*	*(33±2)*	*(19±2)*
57		10±0	11±1	0.9	*(68±2)*	*(90±1)*	*(80±5)*	*(44±1)*	*(58±1)*	*(64±1)*	*(42±7)*	*(66±5)*	*(51±1)*
58		*(24±9)*	*(68±6)*	–	*(9±4)*	*(15±1)*	*(19±5)*	*(2±2)*	*(2±4)*	*(13±6)*	*(<0)*	*(11±3)*	*(11±7)*
59		43±5.5	27±6.6	1.59	*(7±16)*	*(14±1)*	*(11±4)*	*(12±3)*	*(13±1)*	*(16±3)*	*(0.4±1)*	*(15±8)*	*(18±7)*
60		*(62±7)*	*(84±5)*	–	*(20±7)*	*(7±3)*	*(14±4)*	*(12±3)*	*(7±3)*	*(7±2)*	*(2±3)*	*(13±4)*	*(8±3)*
61		(23±4)	(63±19)	–	*(19±7)*	*(21±2)*	*(12±3)*	*(6±2)*	*(16±4)*	*(17±4)*	*(5±4)*	*(23±2)*	*(19±4)*
62		8.2±2.5	8.2±0.7	1	28±2	32±0.3	32±4	66±1	45±2	38±4	41±5	51±5	59±4
63		14±2	15±1	0.9	*(56±2)*	*(79±1)*	*(64±10)*	*(29±2)*	*(61±2)*	*(51±8)*	*(37±6)*	*(39±5)*	*(56±3)*
64		(73±1)	(79±4)	–	*(38±2)*	*(26±3)*	*(51±18)*	*(21±3)*	*(17±2)*	*(17±4)*	*(5±4)*	*(19±6)*	*(26±2)*
65		(43±9)	(40±11)	–	*(40±6)*	*(50±3)*	*(41±1)*	*(38±2)*	*(35±4)*	*(68±2)*	*(36±4)*	*(39±1)*	*(44±4)*

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50} μM) or cell growth by 50% (GI_{50} μM) relative to an untreated control. [b] Values in (*italics*) are percentage enzyme or growth inhibition at 100 μM drug concentration.

Table 6. Protein phosphatase 1 and 2A inhibition and growth inhibition of compounds **66–74**.[a,b]

Compound		Enzyme Inhibition IC_{50} [μM] or [%]				Cell Line Growth Inhibition GI_{50} [μM] or [%]							
		PP1	PP2A	PP2A/PP1	HT29 Colon	SW480 Colon	MCF-7 Breast	A2780 Ovarian	H460 Lung	A431 Skin	DU145 Prostate	BE2-C Neuronal	SJ-G2 Glial
66		34±3.5	7.6±0.4	4.5	*(42±5)*	*(51±6)*	*(39±2)*	*(33±5)*	*(41±2)*	*(50±6)*	*(41±3)*	*(60±10)*	*(63±3)*
67		35±0	12±0	2.9	*(75±1)*	*(95±3)*	*(90±4)*	63±3	35±1	42±2	52±2	40±3	55±8
68		35±5	13±1	2.7	*(60±1)*	*(89±2)*	*(74±3)*	*(56±1)*	*(82±9)*	*(81±1)*	*(69±3)*	*(94±5)*	*(85±2)*
69		38±2	12±0	3.2	*(77±1)*	*(95±2)*	*(79±7)*	*(72±4)*	*(87±5)*	*(87±3)*	*(76±3)*	*(>100)*	*(83±2)*
70		*(63±3)*	*(86±2)*	–	*(59±0.3)*	*(77±5)*	*(36±3)*	*(36±4)*	*(68±8)*	*(58±5)*	*(41±10)*	*(59±6)*	*(47±4)*
71		–[c]	–[c]	–	–[c]	–	–	–	–	–	–	–	–
72		33±0.4	29±2	1.1	47±3	31±1	61±3	63±3	35±1	42±2	52±2	40±3	55±8
73		25±0.0	25±4	1.0	40±3	26±3	56±2	53±2	32±1	37±1	43±0	32±3	48±5
74		*(68±3)*	*(87±2)*	–	52±5	40±4	71±5	65±4	53±4	50±2	66±0	51±5	61±8

[a] Data is expressed as the drug concentration required to inhibit enzyme activity by 50% (IC_{50}; μM) or cell growth by 50% (GI_{50}; μM) relative to an untreated control. [b] Values in *(italics)* are percentage enzyme or growth inhibition at 100 μM drug concentration. [c] Insoluble in testing medium.

aluminium backed silica gel plates with fluorescent indicator (Merck 60 F_{254}). Unless otherwise noted, NMR spectra were recorded in $CDCl_3$ at 300 (^{1}H) or 75 MHz (^{13}C) on a Bruker Advance 300MX spectrometer. GCMS was performed using a Shimadzu GCMS-QP2010 instrument, using a quadrupole mass spectrometer with electron ionisation (EI) or chemical ionisation using methane (CI). HRMS spectra were recorded at the University of Wollongong Biomolecular Mass Spectrometry Laboratory, using a VG Autospec-oa-TOF tandem high resolution mass spectrometer with CI using methane, and PFK as the reference.

Cell culture and stock solutions: Stock solutions were prepared as follows and stored at −20 °C: Cantharidin (Biomol, USA) as a 30 mM solution in DMSO, norcantharidin as a 30 mM solution in H_2O, and norcantharidin analogues as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (1001 IU mL^{-1}), streptomycin (100 μg mL^{-1}), and glutamine (4 mM).

In vitro growth inhibition assays: Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in medium (100 mL) at a density of 2500–4000 cells/well. On day 0 (24 h after plating), when the cells were in logarithmic growth, medium (100 μL) with or without the test agent was added to each well. After 72 h of drug exposure, growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and their absorbance was read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 100 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis to allow the calculation of GI_{50} values. The GI_{50} value is defined as the drug concentration at which cell growth is 50% inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure.[23]

Chemistry

General synthetic procedure: An amine (1 equiv, 2.97 mmol) was added to a solution of norcantharidin **2** (1.0 g, 2.97 mmol) in THF (10 mL) and stirred at RT for 16 h. The reaction was concentrated in vacuo and diluted with acetone (~100 mL). The resulting precipitate was either recrystallised (EtOAc/hexanes, 1:1) or purified by flash chromatography (MeOH/CH_2Cl_2, ~20%) to afford the desired product (30–90%).

3-Propylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (14): ^{1}H NMR ([D$_6$]DMSO): $\delta = 0.80$ (t, $J = 7.5$ Hz, 3H), 1.34–1.36 (m, 2H), 1.41–1.51 (m, 4H), 2.81 (s, 2H), 2.90 (q, $J = 5.8$ Hz), 4.45 (d, $J = 3.1$ Hz, 1H), 4.71 (d, $J = 2.0$ Hz, 1H), 7.23 ppm (d, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta = 11.0$, 22.2, 28.3, 28.7, 40.3,

51.4, 53.1 , 76.7, 78.7, 170.2, 172.2 ppm; mp: 125 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{11}H_{18}NO_4$: 228.1230, found 228.1239

3-Butylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (15): ^{1}H NMR ([D$_6$]DMSO): $\delta = 0.84$ (t, $J = 7.2$ Hz, 3H), 1.26–1.34 (m, 4H), 1.46–1.52 (m, 4H), 2.81 (s, 2H), 2.96 (q, $J = 5.9$ Hz, 2H), 4.45 (d, $J = 4.0$ Hz, 1H), 4.71 (d, $J = 3.6$ Hz, 1H), 7.24 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta = 13.5$, 19.4, 28.3, 28.7, 31.0, 51.5, 53.1, 76.7, 78.7, 170.2, 172.2 ppm; mp: 96–98 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{12}H_{20}NO_4$: 242.1387, found 242.1393.

3-Hexylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (16): ^{1}H NMR (CDCl$_3$): $\delta = 0.87$ (3H, t), 1.27 (8H, m), 1.30 (2H, m), 1.57 (2H, m), 1.80 (2H, m), 3,03 (2H, q), 3.17 (2H, m), 4.67 (1H, d $J = 4.8$ Hz), 5.03 (1H, d $J = 4.6$ Hz), 6.64 ppm (1H, t $J = 5.5$ Hz); ^{13}C NMR (CDCl$_3$): $\delta = 13.4$, 21.9, 25.7, 28.4, 26.9, 28.8, 30.7, 38.5, 51.4, 53.0, 76.7, 78.7, 170.3, 172.3 ppm; mp: 126–128 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{24}NO_4$: 270.1700, found 270.1705.

3-Octylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (17): 1H NMR ([D$_6$]DMSO): $\delta = 1.22$–1.24 (m, 11H), 1.49–1.60 (m, 6H), 1.82–1.85 (m, 2H), 2.83 (s, 2H), 3.43 (t, $J = 7.5$ Hz), 4.84 ppm (q, $J = 2.1$ Hz, 2H); ^{13}C NMR ([D$_6$]DMSO): $\delta = 26.0$ (CH_3), 27.0 (CH_2), 28.0 (2×CH_2), 28.5 (CH2), 28.8 (CH_2), 28.9 (CH_2), 38.5 (CH_2), 49.3 (CH), 49.3 (CH), 78.5 (CH_2), 176.6 ppm (2×C=O) ; mp: 131 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{28}NO_4$: 298.2013, found 298.2014.

3-Decylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (18): ^{1}H NMR ([D$_6$]DMSO): $\delta = 0.84$ (t, $J = 5.6$ Hz, 3H), 1.22 (m, 16H), 1.28–1.54 (m, 4H), 2.80 (m, 2H), 2.94 (t, $J = 5.6$ Hz, 2H), 4.43 (d, $J = 3.9$ Hz, 1H), 4.71 (d, $J = 2.6$ Hz, 1H), 7.33 (t, $J = 5.6$ Hz, 1H), 12.01 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 13.8$, 22.0, 26.4, 28.3, 28.6, 28.7, 28.8, 28.9, 31.2, 38.4, 51.3, 52.9, 76.2, 78.7, 170.2, 172.3 ppm; mp: 110–111 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{32}NO_4$: 326.2326, found 326.2323.

3-Dodecylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (19): ^{1}H NMR (CDCl$_3$): $\delta = 6.67$ (1H, t, $J = 5.3$ Hz), 5.03 (1H, d, $J = 3.5$ Hz), 4.66 (1H, d, $J = 4.3$ Hz), 3.18–3.00 (4H, m), 1.80 (2H, m), 1.57 (2H, m), 1.45 (2H, m), 1.25 (20H, s), 0.87 ppm (3H, t); ^{13}C NMR: $\delta = 172.3$, 170.3, 78.7, 76.7, 53.0, 51.4, 38.5, 30.7, 29.0, 28.9, 28.8, 28.5 28.6, 28.4, 26.9, 25.7, 21.9,13.4 ppm; mp: 115–117 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{20}H_{36}NO_4$: 354.2639, found 354.2644.

3-Tetradecylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (20): ^{1}H NMR (CDCl$_3$): $\delta = 0.87$ (3H, t), 1.24 (22H, s), 1.44 (2H, m), 1.54 (2H, m), 1.78 (2H, m), 3.01–3.16 (4H, m), 4.65(1H, d), 5.01-(1H, d), 6,76 ppm (1H, t); ^{13}C NMR (CDCl$_3$): $\delta = 13.0$, 21.9, 25.7, 26.9, 28.4, 28.5, 28.6, 28.8, 28.9, 29.0, 30.7, 38.5, 51.4, 53.0, 76.7, 78.7, 170.3, 172.3 ppm; mp: 123–125 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{22}H_{40}NO_4$: 382.2952, found 382.2955.

3-Octadecylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (21): ^{1}H NMR (CDCl$_3$): $\delta = 0.87$ (3H, t), 1.25 (30H, s), 1.45 (2H, m), 1.55 (2H, m), 1.80 (2H, m), 3.17–3.01 (4H, m), 4.65 (1H, d), 5.00 (1H, d), 6.71 ppm (1H, t); ^{13}C NMR (CDCl$_3$): $\delta = 13.4$, 21.9, 25.7, 26.9, 28.4, 28.5 28.6, 28.8, 28.9, 29.0, 30.7, 38.5, 51.4, 53.0, 76.7, 78.7, 170.3, 172.3 ppm; mp: 124–126 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{26}H_{48}NO_4$: 438.3578, found 438.3587.

3-Allylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (22): ^{1}H NMR (CDCl$_3$): $\delta = 1.46$–1.52 (m, 4H), 2.91 (d, $J = 5.1$ Hz, 1H), 2.16 (d, $J = 5.1$ Hz, 1H), 3.72 (m, 2H), 4.67 (t, $J = 5.2$ Hz, 2H), 5.06–5.13 (m, 2H), 5.76–5.82 (m, 1H), 8.14 ppm (br s, 1H); ^{13}C NMR (CDCl$_3$): $\delta = 24.5$, 29.0, 50.0, 51.8, 77.6, 79.6, 115.0, 135.2 , 168.9, 173.0, 173.8 ppm; mp: 105–107 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{11}H_{16}NO_4$: 226.1074, found 226.1076.

3-(3-Carboxpropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (23): ^{1}H NMR (CDCl$_3$): $\delta = 1.52$ (4H, m), 2.16 (2H, t), 2.78 (2H, t), 3.02 (2H, m), 4.47 (1H, d), 4.72 (1H, d), 7.25 ppm (1H, t); ^{13}C NMR (CDCl$_3$): $\delta = 24.8$, 28.3, 28.7, 31.7, 37.9, 52.3, 53.3, 76.9, 78.3, 170.9, 172.8, 174.7 ppm; mp: 135–137 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{12}H_{18}NO_6$: 272.1129, found 272.1131.

3-(5-Carboxypentylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (24): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.34$ (4H, m), 1.47 (2H, m), 2.16 (2H, t $J = 7.3$ Hz), 2.96 (2H, q $J = 6.0$ Hz), 4.46 (2H, d, $J = 4.0$ Hz), 4.71 (1H, d $J = 3.5$ Hz), 7.28 ppm (1H, t); ^{13}C NMR ([D$_6$]DMSO): $\delta = 24.2$, 25.9, 28.3, 28.4, 28.6, 38.2, 38.9, 51.6, 53.1, 76.72, 78.6, 170.3, 172.3, 174.5 ppm; mp: 98–100 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{22}NO_6$: 300.1442, found 300.1447.

3-(7-Carboxyheptylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (25): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.23$ (6H, m), 1.51–1.46 (4H, m), 2.17 (2H, t), 2.81 (2H, t), 2.96 (2H,q), 4.45 (1H, d), 4.70 (1H, d), 7.27 ppm (1H, t); ^{13}C NMR ([D$_6$]DMSO): $\delta = 24.4$, 26.2, 28.4, 28.7, 28.8, 33.6, 38.4, 51.4, 53.0, 76.7, 78.7, 170.2, 172.3, 174.4 ppm; mp: 125–126 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{26}NO_6$: 328.1755, found 328.1759.

3-(6-Hydroxyhexylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (26): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.43$ (10H, m), 2.75 (2H, m), 2.94 (2H, quin, $J = 5.64$ Hz), 3.35 (2H, t, $J = 6.33$), 4.43 (1 h, d, $J = 4.23$ Hz), 4. 67 (1H, d), 7. 27 ppm (1H, t, $J = 5$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta = 25.1$, 25.8, 28.5, 28.7, 28.9, 32.2, 32.4, 38.4, 53.3, 53.54, 60.49, 77.3,78.4, 171.1, 173.4 ppm; mp: 47–49 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{24}NO_5$: 286.1649, found 286.1655.

3-[2-(3 H-Imidazol-4-yl)-ethylcarbamoyl]-7-oxa-bicyclo-[2.2.1]heptane-2-carboxylic acid (28): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1$–41–1.46 (4H, m), 2.58 (2H, m), 2.82 (2H, m), 3.18 (2H, m), 4.45 (1H, d), 4.70 (1H, d), 6.77(1H, t), 7.35 (1H, m), 7.50 ppm (1H, m); ^{13}C NMR ([D$_6$]DMSO): $\delta = 23.9$, 27.9, 28.30, 49.3, 51.6, 53.10, 78.27, 78.55, 116.6, 134.7, 143.1,170.4, 172.4 ppm; mp: 145–147 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{13}H_{18}N_3O_4$: 280.1292, found 280.1290.

3-Phenylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (31): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.48$–1.59 (m, 4H), 2.92 (d, $J = 9.4$ Hz, 1H), 3.03 (d, $J = 9.4$ Hz, 1H), 4.62 (d, $J = 3.9$ Hz, 1H), 4.76 (s, 1H), 7.01 (t, $J = 6.7$ Hz, 1H), 7.25 (t, $J = 7.6$ Hz, 1H), 7.50 (d, $J = 7.6$ Hz, 1H), 9.64 ppm (s, 1H, NH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 29.3$, 29.8, 52.6, 54.4, 77.8, 79.6, 120.1 (2C), 123.8, 129.4 (2C), 140.1, 170.1, 173.1 ppm; mp: 170 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{16}NO_4$: 262.1074, found 262.1079.

3-(2-Chlorophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (32): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.55$–1.66 (m, 4H), 3.12 (dd, $J = 13.9$ and 9.9 Hz, 2H), 4.75 (d, $J = 4.3$ Hz, 1H), 4.88 (s, 1H), 7.08 (t, $J = 7.6$ Hz, 1H), 7.28 (t, $J = 7.2$ Hz, 1H), 7.43 (d, $J = 7.9$ Hz, 1H), 8.03 (d, $J = 7.9$ Hz, 1H), 9.02 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta = 29.2$, 29.4, 52.9, 55.3, 123.7, 124.3, 125.7, 128.3, 130.0, 136.0, 170.6, 173.0 ppm; mp: 135–136 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{15}ClNO_4$: 296.0684, found 296.0690.

3-(3-Chlorophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (33): ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.48$–1.64 (m, 4H), 2.94

(d, J=9.6 Hz, 1H), 3.03 (d, J=9.6 Hz, 1H), 4.62 (d, J=3.8 Hz, 1H), 4.76 (s, 1H), 7.06 (dd, 2.0 and 1.6 Hz, 1H), 7.28 (t, J=8.1 Hz, 1H), 7.76 (d, J=1.6 Hz, 1H), 9.86 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ= 29.3, 29.8, 52.7, 54.3, 77.8, 79.4, 118.4, 119.6, 123.5, 131.1, 133.8, 140.5, 170.6, 173.0 ppm; mp: 163–164 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{15}ClNO_4$: 296.0684, found 296.0687.

3-(4-Bromophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (34): ^{1}H NMR: ([D$_6$]DMSO): δ=1.45–1.60 (4H, m), 2.93 (1H, d J=9.3 Hz), 3.03 (1H, d J=9.6 Hz), 4.63 (1H, d J=3.1 Hz), 4.76 (1H, d J=3.3 Hz), 7.42–7.51(4H, q J=8.7 Hz), 9.78 (1H, s), 11.96 ppm (1H, s); ^{13}C NMR: ([D$_6$]DMSO): δ=28.3, 28.9, 51.6, 53.3, 76.8, 78.5, 114.3, 121.0, 131.3, 138.5, 169.4, 172.0 ppm; mp: 187–189 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{15}BrNO_4$: 340.0179, found 340.0186.

3-(3-Iodophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (35): ^{1}H NMR: ([D$_6$]DMSO): δ=1.48–1.60 (4H, m), 2.91 (1H, d J=9.6 Hz), 3.00 (1H, d J=9.6 Hz), 4.63(1H, d J=3.9 Hz), 4.76 (1H, d J=2.7 Hz), 7.10 (1H, t J=8.1 Hz), 7.41–7.34 (2H, m), 8.08 (1H, d J=1.5 Hz), 9.77 (1H, s), 11.96 ppm (1H, s); ^{13}C NMR: ([D$_6$]DMSO): δ=28.3, 28.9, 51.6, 53.3, 76.8, 78.5, 94.3, 118.3, 127.4, 130.5, 131.4, 140.6, 169.5, 172.1 ppm; mp: 192–193 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{15}INO_4$: 388.0040, found 388.0047.

3-(4-Iodophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (36): ^{1}H NMR ([D$_6$]DMSO): δ=1.52–1.56 (m, 4H), 2.92 (d, J=9.6 Hz, 1H), 3.02 (d, J=9.6 Hz, 1H), 4.62 (s, 1H), 4.76 (s, 1H), 7.35 (d, J=8.7 Hz, 1H), 7.59 (d, J=8.7 Hz, 1H), 9.74 (s, 1H), 11.9 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=28.3, 28.9, 51.6, 53.4, 76.8, 78.5, 86.0, 121.3 (2C), 137.1 (2C), 139.0, 169.4, 172.0 ppm; mp: 184 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{15}INO_4$: 388.0040, found 388.0042.

3-(4-Nitrophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (37): ^{1}H NMR ([D$_6$]DMSO): δ=1.50–1.58 (m, 4H), 2.99 (d, J=9.3 Hz, 1H), 3.09 (d, J=9.3 Hz, 1H), 4.64 (s, 1H), 4.75 (s, 1H), 7.76 (d, J=8.7 Hz, 2H), 8.17 (d, J=8.7 Hz, 2H), 10.33 (s, 1H), 12.01 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=29.3, 29.8, 52.8, 54.3, 119.5 (2C), 125.7 (2C), 142.8, 146.4, 171.1, 172.9 ppm; mp: 154 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{15}N_2O_6$: 307.0925, found 307.0930.

3-(2-Hydroxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (38): ^{1}H NMR ([D$_6$]DMSO): δ=1.52–1.65 (m, 4H), 3.04 (d, J=9.9 Hz, 1H), 3.11 (d, J=9.9 Hz, 1H), 4.67 (d, J=4.2 Hz, 1H), 4.84 (s, 1H), 6.71 (t, J=7.8 Hz, 1H), 6.78–6.85 (m, 2H), 7.89 (d, J=7.8 Hz, 1H), 8.88 (s, 1H), 9.65 (br s, 1H), 12.1 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=28.3, 28.4, 51.9, 54.6, 76.9, 78.9, 114.8, 118.7, 120.3, 123.4, 126.7, 146.6, 169.5, 171.9 ppm; mp: 238–239 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{16}NO_5$: 278.1023, found 278.1029.

3-(4-Hydroxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (39): ^{1}H NMR ([D$_6$]DMSO): δ=1.47–1.56 (m, 4H), 2.89 (d, J=9.6 Hz, 1H), 2.99 (d, J=9.6 Hz, 1H), 4.59 (d, J=3.8 Hz, 1H), 4.75 (s, 1H), 6.63 (d, J=8.7 Hz, 2H), 7.25 (d, J=8.7 Hz, 2H), 9.06 (s, 1H), 9.26 ppm (br s, 1H); ^{13}C NMR: ([D$_6$]DMSO): δ=28.3, 28.9, 51.5, 53.4, 76.7, 78.6, 114.8 (2C), 121.0 (2C), 130.7, 153.1, 168.5, 172.1 ppm; mp: 154–156 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{14}H_{16}NO_5$: 278.1023, found 278.1029.

3-(4-Carboxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (40): ^{1}H NMR ([D$_6$]DMSO): δ=1.49–1.60 (4H, m), 2.95 (1H, d J=9.5 Hz), 3.06 (1H, d J=9.5 Hz), 4.63 (1H, d J=3.5 Hz), 4.76 (1H, d J=2.1 Hz), 7.61 (2H, d J=8.5 Hz), 7.85 (2H, d J=8.5 Hz), 9.98 ppm (s, 1H); ^{13}C NMR: ([D$_6$]DMSO): δ=28.3, 28.8, 51.7, 53.4, 76.9, 78.5, 118.3, 124.8, 130.2, 143.2, 166.9, 169.8, 172.0 ppm; mp: 271–273 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{16}NO_6$: 306.0972, found 306.0977.

3-(4-Methoxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (41): ^{1}H NMR ([D$_6$]DMSO): δ=1.51–1.56 (m, 4H), 2.90 (d, J=9.6 Hz, 1H), 3.09 (d, J=9.6 Hz, 1H), 3.69 (s, 1H), 4.60 (d, J=3.7 Hz, 1H), 4.76 (d, J=3.7 Hz, 1H), 6.83 (d, J=8.9 Hz, 2H), 7.40 (d, J=8.9 Hz, 2H), 9.39 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=29.3, 29.8, 52.5, 54.4, 56.1, 77.8, 79.6, 114.6, 121.7, 133.3, 156.0, 169.7, 173.1 ppm; mp: 153–154 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_5$: 292.1179, found 292.1184.

3-(4-Thiomethylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (42): ^{1}H NMR ([D$_6$]DMSO): δ=1.48–1.60 (m, 4H), 2.42 (s, 3H), 2.92 (d, J=9.6 Hz, 1H), 3.03 (d, J=9.6 Hz, 1H), 4.62 (d, J=3.9 Hz, 1H), 4.84 (d, J=3.9 Hz, 1H), 7.19 (d, J=8.4 Hz, 2H), 7.47 (d, J=8.4 Hz, 2H), 9.61 (s, 1H), 11.9 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=16.7, 29.3, 29.8, 52.6, 54.3, 77.7, 79.5, 120.8 (2C), 128.1 (2C), 132.2 , 137.8, 170.1, 173.0 ppm; mp: 165–166 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_4S$: 308.0951, found 308.0947.

3-(2-Thiomethylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (43): ^{1}H NMR ([D$_6$]DMSO): δ=1.57 (m, 4H), 2.37 (s, 3H), 3.09 (s, 2H), 4.75 (d, J=4.2 Hz, 1H), 4.88 (s, 1H), 7.08 (t, J=7.5 Hz, 2H), 7.19 (t, J=7.5 Hz, 1H), 7.38 (d, J=7.8 Hz, 1H), 7.84 (d, J=7.8 Hz, 1H), 8.98 (s, 1H), 12.2 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=16.5, 28.3, 28.4, 51.8, 54.6, 77.0, 78.9, 122.2, 124.5, 126.6, 128.2, 129.7 , 137.1, 169.6, 172.0 ppm; mp: 118–120 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_4S$: 308.0951, found 308.0951.

3-(4-Pentoxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (44): ^{1}H NMR ([D$_6$]DMSO): δ=0.88 (t, J=6.9 Hz, 3H), 1.34–1.36 (m, 4H), 1.51–1.67 (m, 6H), 2.91 (d, J=9.6 Hz, 1H), 3.01 (d, J=9.6 Hz, 1H), 3.89 (t, J=6.5 Hz, 2H), 4.61 (d, J=3.8 Hz, 1H), 4.84 (d, J=3.8 Hz, 1H), 6.82 (d, J=8.9 Hz, 2H), 7.39 (d, J=8.9 Hz, 1H), 9.41 (s, 1H), 10.85 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ= 14.8, 22.8, 28.6, 29.3 (2C), 29.8, 52.5, 54.3, 68.5, 77.7, 79.6, 115.2 (2C), 121.7 (2C), 133.2, 155.4, 169.7, 173.1 ppm; mp: 156 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{19}H_{26}NO_5$: 348.1805, found 348.1821.

3-(4-Octyloxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (45): ^{1}H NMR ([D$_6$]DMSO): δ=0.82 (t, J=7.0 Hz, 3H), 1.24 (br s, 8H), 1.45–1.70 (m, 4H), 1.75 (qn, J=3.0 Hz, 3H), 2.95 (q, J=3.1 Hz, 2H), 3.58 (qn, J=2.5 Hz, 4H), 3.86 (t, J=6.5 Hz, 2H), 4.59 (d, J=4.0 Hz, 1H), 4.75 (d, J=2.7 Hz, 1H), 6.81 (d, J=9.0 Hz, 1H), 7.38 (d, J=9.0 Hz, 1H), 9.49 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=14.8, 22.9, 26.0, 26.4, 29.5, 29.6, 32.1, 52.5, 54.3, 67.9, 68.5, 77.7, 79.6, 115.3, 121.7, 133.2, 155.4, 169.7, 173.1 ppm; mp: 132 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{22}H_{32}NO_5$: 390.2275, found 390.2279.

3-(2-Benzylalcoholcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (46):^{1}H NMR ([D$_6$]DMSO): δ=1.51–1.62 (m, 4H), 3.03 (t, J=10.3 Hz, 2H), 4.45 (s, 2H), 4.69 (d, J=4.2 Hz, 1H), 4.08 (s, 1H), 5.22 (br s, 1H), 7.06 (t, J=7.1 Hz, 1H), 7.19 (t, J=7.1 Hz, 1H), 7.32 (d, J=7.4 Hz, 1H), 7.63 (d, J=7.9 Hz, 1H), 8.99 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ=29.0, 29.1, 52.4, 54.7, 60.7, 77.6, 79.3, 123.5, 124.4, 127.4, 127.9, 133.9, 136.4, 169.9, 172.7 ppm; mp: 145–146 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_5$: 292.1179, found 292.1175.

3-(4-Benzylalcoholcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (47): ^{1}H NMR ([D$_6$]DMSO): δ = 1.50–1.65 (m, 4H), 2.97 (dd, J = 26.4 Hz, 2H), 4.39 (d, J = 3.9 Hz, 2H), 4.61 (d, J = 3.9 Hz, 1H), 4.75 (d, J = 2.2 Hz, 1H), 5.07 (br s, 1H), 7.19 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 9.62 ppm (s, 1H). ^{13}C NMR ([D$_6$]DMSO): δ = 29.3, 29.9 , 52.6, 54.5, 63.6, 77.8, 79.6, 119.9 (2C), 127.7 (2C), 138.0, 138.7 , 170.1, 173.1 ppm; mp: 137–138 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_5$: 292.1179, found 292.1179.

3-(3-Benzylalcoholcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (48): ^{1}H NMR ([D$_6$]DMSO): δ = 1.49–1.60 (m, 4H), 2.92 (d, J = 9.6 Hz, 1H), 3.05 (d, J = 9.6 Hz, 1H), 4.44 (s, 2H), 4.62 (d, J = 3.8 Hz, 1H), 4.75 (d, J = 3.8 Hz, 1H), 5.11 (br s, 1H), 7.76 (d, J = 7.4 Hz, 1H), 7.19 (t, J = 7.7 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 9.54 (s, 1H), 10.9 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 29.3, 29.8, 52.5, 54.5, 63.7, 77.8, 79.6, 118.2, 118.4, 121.9, 129.1, 140.0, 143.9 , 170.1, 173.1 ppm; mp: 156–157 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{15}H_{18}NO_5$: 292.1179, found 292.1175.

3-(3-Styrylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (49): ^{1}H NMR ([D$_6$]DMSO): δ = 1.48–1.60 (m, 4H), 2.92 (d, J = 9.6 Hz, 1H), 3.04 (d, J = 9.6 Hz, 1H), 4.62 (d, J = 3.8 Hz, 1H), 4.76 (s, 1H), 5.13 (d, J = 11.1 Hz, 1H), 5.68 (d, J = 17.7 Hz, 1H), 6.85 (dd, J = 17.7 and 11.1 Hz), 7.36 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 9.68 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 29.2, 29.6, 52.6, 54.4, 77.7, 79.3, 113.2, 120.0 (2C), 127.1 (2C), 132.8, 137.0, 139.7, 169.9, 172.7 ppm; mp: 157 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{18}NO_4$: 288.1230, found 288.1244.

3-(3-Ethynylphenycarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (50): ^{1}H NMR ([D$_6$]DMSO): δ = 1.52–1.54 (m, 4H), 2.93 (d, J = 9.6 Hz, 2H), 3.03 (d, J = 9.6 Hz, 2H), 4.10 (s, 1H), 4.63 (d, J = 3.8, 1H), 4.76 (s, 1H), 7.11 (d, J = 7.3 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.73 (s, 1H), 9.75 (s, 1H), 10.9 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 28.3, 28.8, 51.8, 53.5, 77.0, 78.4, 79.4, 83.3, 119.9, 121.8, 122.3, 126.1, 128.8, 139.3, 169.4, 171.9 ppm; mp: 166 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{16}NO_4$: 286.1074, found 286.1083.

3-(4-Carboxyethylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (51): ^{1}H NMR ([D$_6$]DMSO): δ = 1.49–1.57 (4H, m), 2.92 (1H, d J = 9.6 Hz), 3.03 (1H, d J = 9.6 Hz), 3.47 (2H, s), 4.62 (1H, d J = 4.2 Hz), 4.76 (1H, d J = 3.1 Hz), 7.13 (2H, d J = 8.4 Hz), 7.44 (2H, d J = 8.7 Hz), 9.55 (1H, NH), 12.04 ppm (1H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 28.3, 28.9, 51.6, 53.4, 76.8, 78.6, 119.0, 129.3, 129.4, 137.7, 169.1, 172.1, 172.7 ppm; mp: 268–270 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{18}NO_6$: 320.1129, found 320.1140.

3-(3-Carboxyethylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (52): ^{1}H NMR ([D$_6$]DMSO): δ = 1.49–1.57 (4H, t), 2.91 (2H, d J = 9.6 Hz), 3.05 (2H, d J = 9.6 Hz), 3.49 (2H, s), 4.61(1H, d J=3.9 Hz), 4.76 (1H, d J = 3.0 Hz), 6.89 (1H, d J = 7.5 Hz), 7.19 (1H, t J = 7.5 Hz), 7.39 (1H, d J = 8.1 Hz), 7.46 (1H, s), 9.63 (1H,s NH), 11.99 ppm (1H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 28.4, 28.9, 40.8, 51.5, 53.5, 76.8, 78.7, 117.4, 119.8, 123.9, 128.4, 135.3, 139.2, 169.2, 172.1, 172.4 ppm; mp: 144–146 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{18}NO_6$: 320.1129, found 320.1136.

3-(3-Carboxypropylphenylcarbamoyl)-7-oxabicyclo-[2.2.1]heptane-2-carboxylic acid (53): ^{1}H NMR ([D$_6$]DMSO): δ = 1.52–1.56 (4H, m), 2.49(2H, t), 2.74 (2H, t), 2.93 (1H, d), 3.01 (1H, d), 4.61 (1H, 4.2 Hz), 4.76 (1H, 3.3 Hz), 7.10 (2H, d J = 8.4 Hz), 7.39 (2H, d J = 8.4 Hz), 9.50 (1H, s NH), 11.97 ppm (2H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 28.3, 28.8, 29.7, 35.3, 51.6, 53.5, 76.8, 78.6, 119.2, 128.2, 135.4, 137.1, 169.0, 172.1, 173.6 ppm; mp: 189–191 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{17}H_{20}NO_6$: 334.1285, found 334.1288.

3-(4-Morphilinophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (54): ^{1}H NMR ([D$_6$]DMSO): δ = 1.47–1.56 (m, 4H), 2.88–3.02 (m, 6H), 4.60 (d, J = 3.7 Hz, 1H), 4.76 (d, J = 2.1 Hz, 1H), 6.85 (d, 8.9 Hz, 1H), 7.36 (d, J = 8.9 Hz, 1H), 9.35 (s, 1H), 10.9 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 29.3, 29.8, 50.0, 52.5, 54.4, 67.0, 77.7, 79.6 (CH), 116.3 (2C), 121.2 (2C), 132.5, 148.0, 169.6, 173.1 ppm; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{18}H_{23}N_2O_5$: 347.1601, found 347.1614.

3-(2-Ethylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (55): ^{1}H NMR ([D$_6$]DMSO): δ = 1.53–1.67 (m, 4H), 2.50 (s, 3H), 3.05 (d, J = 9.6 Hz, 1H), 3.10 (d, J = 9.6 Hz, 1H), 4.72 (d, J = 3.6 Hz, 1H), 4.84 (d, J = 2.8 Hz, 1H), 7.01–7.18 (m, 3H), 7.65 (d, J = 8.8 Hz, 1H), 8.72 (br s, 1H), 12.07 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 23.5, 28.38, 28.43, 51.7, 54.1, 77.0, 79.0, 123.2, 124.4, 125.8, 128.2, 135.1, 135.7, 169.4, 172.2 ppm; mp: 159–160 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{20}NO_4$: 290.1387, found 290.1390.

3-(2-*tert*-Butylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (56): ^{1}H NMR ([D$_6$]DMSO): δ = 1.32 (9 H, s), 1.54–1.60 (4 H, m), 3.08 (2H, dd, J = 13.6 and 10.0 Hz), 4.69 (1H, d, J = 4.5 Hz), 4.84 (1H, s), 7.12–7.15 (2H, m), 7.28–7.35 (2H, m), 8.53 (1H, s), 11.91 (1H, brs) ppm; ^{13}C NMR ([D$_6$]DMSO): δ = 29.3, 29.5, 31.3 (3C), 35.2, 52.6, 54.7, 77.9, 79.3, 126.6, 126.9; 127.0, 130.0, 136.7, 144.7, 170.5, 173.2 ppm; mp: 163–164 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{18}H_{23}NO_4$: 318.1661, found 318.1706.

3-(4-*tert*-Butylphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (57): ^{1}H NMR ([D$_6$]DMSO): δ = 1.24 (9 H, s), 1.45–1.60 (4 H, m), 2.91 (1H, d, J = 9.6 Hz), 3.03 (1H, d, J = 9.6 Hz), 4.61 (1H, d, J = 3.3 Hz), 4.77 (1H, s), 7.26 (2H, d, J = 8.4 Hz), 7.42 (2H, d, J = 8.4 Hz), 9.48 (1H, s), 11.89 (1H, brs) ppm; ^{13}C NMR ([D$_6$]DMSO): δ = 29.3, 29.8, 32.0 (3C), 34.8, 52.5, 54.4, 119.7, 119.8, 126.0, 137.5, 146.1, 169.9, 173.1 ppm; mp: 179 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{18}H_{23}NO_4$: 318.1661, found 318.1700.

3-(2′,6′-Dimethylphenylcarbamoyl)7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (58): ^{1}H NMR ([D$_6$]DMSO): δ = 1.52–1.59 (4H, m), 2.13 (6H, s), 2.90 (1H, d, J = 9.6 Hz), 3.15 (1H, d, J = 9.6 Hz), 4.60 (1H, d, J = 4.7 Hz), 4.78 (1H, d, J = 3.6 Hz), 7.02 (3H, s), 8.96 (1H, NH), 11.86 ppm (1H, COOH); ^{13}C NMR ([D$_6$]DMSO): δ = 18.1, 28.5, 29.0, 51.0, 52.9, 76.6, 79.3, 126.1, 127.4, 135.1, 135.2, 168.8, 172.2 ppm; mp: 193–195 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{20}NO_4$: 290.1387, found 290.1398.

3-(2′,4′-Dimethylphenylcarbamoyl)7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (59): ^{1}H NMR ([D$_6$]DMSO): δ = 1.52–1.68 (m, 4H), 2.12 (s, 3H), 2.29 (s, 3H), 3.01 (d, J = 9.7 Hz, 1H), 3.07 (d, J = 9.7 Hz, 1H), 4.69 (d, J = 3.7 Hz, 1H), 4.82 (s, 1H), 6.92 (m, 2H), 7.46 (d, J = 8.0 Hz, 1H), 8.63 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 17.2, 20.3, 28.3, 28.5, 51.7, 53.9 , 77.0, 78.9, 123.0, 126.2, 129.4, 130.5 , 133.1, 133.9 , 169.0, 172.2 ppm; mp: 152 °C; HRMS-ESI: *m/z* [*M*+H]$^+$ calcd for $C_{16}H_{20}NO_4$: 290.1387, found 290.1400.

3-(2′,3′-Dimethylphenylcarbamoyl)7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (60): ^{1}H NMR ([D$_6$]DMSO): δ = 1.52–1.59 (m, 4H), 2.05 (s, 3H), 2.22 (s, 3H), 3.01 (d, J = 9.7 Hz, 1H), 3.08 (d, J = 9.7 Hz, 1H), 4.69 (d, J = 4.2 Hz, 1H), 4.81 (s, 1H), 6.92 (d, J = 7.2 Hz, 1H),

7.00 (t, $J=7.7$ Hz, 1H), 7.34 (d, $J=7.7$ Hz, 1H), 8.78 (s, 1H), 11.1 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=14.3$, 21.1, 29.3, 29.6, 52.7, 54.7, 77.9, 79.9, 122.7, 125.9, 126.0, 129.9, 137.2, 137.4, 170.1, 173.2 ppm; mp: 177 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}NO_4$: 290.1387, found 290.1392.

3-(2′,4′,6′-Trimethylphenylcarbamoyl)7-oxa-bicyclo-[2.2.1]heptane-2-carboxylic acid (61): ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 1.51–1.66 (m, 4H), 2.08 (s, 6H), 2.19 (s, 3H), 2.88 (d, $J=9.4$ Hz, 1H), 3.12 (d, $J=9.4$ Hz, 1H), 4.58 (d, $J=4.5$ Hz, 1H), 4.77 (s, 1H), 6.82 (s, 2H), 8.87 (s, 1H), 11.85 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=$ 17.9 (2C), 18.0, 28.5, 29.0, 50.9, 52.9, 76.6, 79.3, 128.0, 132.4, 134.9, 135.0, 168.8, 172.2 ppm; mp: 194–195 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}NO_4$: 304.1543, found 304.1551.

3-(3′,5′-Di-*tert*-butylphenylcarbamoyl)7-oxa-bicyclo-[2.2.1]heptane-2-carboxylic acid (62): ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 1.25 (s, 18H), 1.49–1.62 (m, 4H), 2.89 (d, $J=9.5$ Hz, 2H), 3.04 (d, $J=$ 9.5 Hz, 2H), 4.61 (d, $J=4.0$ Hz, 1H), 4.84 (s, 1H), 7.05 (s, 1H), 7.39 (s, 2H), 9.50 (s, 1H), 10.9 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=$ 29.4, 29.8, 32.1 (6C), 35.3, 52.3, 54.6, 77.8, 79.8, 114.3, 117.5, 139.6, 151.4, 169.9 (CON), 173.2 ppm; mp: 182 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{22}H_{32}NO_4$: 374.2326, found 374.2339.

3-(2′-Naphthylcarbamoyl)7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (63): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.57$–1.68 (m, 4H), 3.09 (d, $J=$ 9.6 Hz, 2H), 3.27 (d, $J=9.6$ Hz, 2H), 4.81 (d, $J=3.8$ Hz, 1H), 4.87 (s, 1H), 7.46–7.54 (m, 3H), 7.69 (m, 1H), 7.92 (m, 1H), 7.90 (m, 1H), 8.02 (m, 1H), 9.49 ppm (m, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=29.4$, 29.6, 52.8, 54.7, 77.1, 79.9, 120.9, 123.0, 125.4, 126.4, 126.7, 126.8, 127.9, 129.0, 134.4, 134.5, 170.7, 173.2 ppm; mp: 178–179 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{18}NO_4$: 312.1191, found 312.1237.

3-(1-Naphthylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (64): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.51$–1.62 (m, 4H,), 2.96 (d, $J=$ 9.9 Hz, 2H), 3.17 (d, $J=9.9$ Hz, 2H), 4.67 (s, 1H), 4.79 (s, 1H), 7.36–7.53 (m, 3H), 7.74–7.83 (s, 3H), 9.82 (s, 1H), 10.95 ppm (br s, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=29.3$, 29.8, 52.7, 54.5, 77.8, 79.6, 116.0, 120.9, 125.3, 127.2, 128.0, 128.3, 129.0, 130.5, 134.3, 137.7, 170.5, 173.1 ppm; mp: 237–238 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{18}NO_4$: 312.1230, found 312.1241.

3-(1,2,3,4-Tetrahydro-1-naphthylcarbamoyl)-7-oxabicyclo-[2.2.1]heptane-2-carboxylic acid (65): ^{1}H NMR ([D$_6$]DMSO): $\delta=2$ diasteroisomers; 1.45–1.83 (m, 20H), 2.68 (q, $J=6.7$ Hz, 4H), 2.82–2.89 (m, 4H), 3.51 (br s, 1H), 4.13 (br s, 1H), 4.51 (dd, $J=13.9$ and 4.1 Hz, 2H), 4.71 (br s, 2H), 7.05–7.23 (m, 10H), 7.48 (d, $J=7.8$ Hz, 1H), 7.73 ppm (d, $J=7.8$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=19.6$, 20.7, 20.9, 26.5, 29.4, 29.6, 29.7, 30.5, 47.1, 47.2, 52.9, 53.0, 53.7, 54.0, 67.9, 77.8, 77.9, 79.5, 78.8, 126.7, 127.4, 128.0, 129.2, 129.4, 129.5, 129.7, 137.8, 137.9, 138.4, 138.5, 171.0 and 171.1, 173.4 and 173.5 ppm; mp: 150 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{22}NO_4$: 316.1543, found 316.1549.

3-[(Pyridin-2′-ylmethyl)-carbamoyl]-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (66): ^{1}H NMR ($CDCl_3$): $\delta=1.4$–1.56 (4H, m), 2.87 (1H, d), 2.96 (1H, d), 4.30 (2H, dq), 4.54 (1H, d), 4.73 (1H, d), 7.22 (1H, t), 7.32 (1H, m), 7.71 (1H, m), 8.03 (1H, m), 8.45 ppm (1H, m); ^{13}C NMR ($CDCl_3$): $\delta=28.7$, 28.9, 53.1, 53.4, 76.9, 78.7, 79.8, 120.9, 122.1, 136.6, 148.5, 158.4, 171.0, 172.6 ppm; mp: 153–155 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{14}H_{17}N_2O_4$: 277.1183, found 277.1198.

3-Benzylcarbamoyl-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (67): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.43$–1.56 (4H, m), 2.84 (1H, d $J=$ 9.6 Hz), 2.92 (1H, d $J=9.7$ Hz), 4.21 (2H, sept $J=6.1$ Hz), 4.50 (1H, d $J=2.4$ Hz), 4.74 (1H, d $J=2.4$ Hz), 7.29–7.21 (5H, m), 7.93 ppm(1H, t $J=5.7$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.3$, 28.85, 42.1, 51.2, 52.9, 76.7,78.7, 126.6,127.1,128.1, 139.4, 170.5, 172.3 ppm; mp: 162–163 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}NO_4$: 276.1230, found 276.1235.

3-(4′-Methoxybenzylcarbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (68): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.42$–1.52 (4H, m), 2.84, (1H,d $J=9.1$ Hz), 2.89 (1H, d $J=8.9$ Hz), 3.71 (3H,s), 4.13 (2H, sept $J=6.5$ Hz), 4.47 (1H, d $J=3.0$ Hz), 4.73(1H, d $J=2.9$ Hz), 6.85 (2H, d $J=8.6$ Hz),7.16 (2H, d $J=8.5$ Hz), 7.86 ppm (1H, t $J=$ 5.6 Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.3$, 28.8, 41.6, 51.2, 52.9, 55.0, 76.7, 78.8, 113.6, 128.5, 131.3, 158.1, 168.9, 172.3 ppm; mp: 144–145 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}NO_5$: 306.1336, found 306.1347.

3-(3′,4′-Dimethoxybenzylcarbamoyl)-7-oxa-bicylco-[2.2.1]heptane-2-carboxylic acid (69): ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 1.43–1.52 (4H, m), 2.83 (1H, d $J=9.7$ Hz), 2.92 (1H, d $J=9.7$ Hz), 3.70 (3H, s), 3.72 (3H, s), 4.15 (2H, dq $J=16.6$ Hz, $J=5.9$ Hz), 4.49 (1H, d $J=3.1$ Hz), 4.73 (1H, d $J=2.8$ Hz), 6.75–6.78 (1H, m), 6.84–6.87 (2H, m), 7.82 ppm (1H, t $J=5.6$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta=$ 28.3, 28.8, 41.9, 51.3, 52.9, 55.3, 55.5, 76.7, 78.8, 111.2, 111.7, 119.3, 131.8, 147.6, 148.6, 170.3, 172.3 ppm; mp: 155–156 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}NO_6$: 336.1442, found 336.1450.

3-(4′-Chlorobenzylcaramoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (70): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.41$–1.56 (4H, m), 2.87 (2H, q $J=9.0$ Hz), 4.19 (2H, m), 4.50 (1H, d $J=4.0$ Hz), 4.73 (1H, d $J=2.8$ Hz), 7.25–7.41 (4H, q), 7.96 ppm (1H, t $J=5.6$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.4$, 28.8, 41.4, 51.4, 52.9, 76.8, 78.7, 128.0, 129.0, 138.6, 170.6, 172.3 ppm; mp: 183–185 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}ClNO_4$: 310.0841, found 310.0855.

3-(4′-Carboxybenzylcarbamoyl)-7-oxa-bicyclo[2.2.1]hepane-2-carboxylic acid (71): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.42$–1.61 (4H, m), 2.89 (2H q $J=11.7$ Hz), 4.26 (2H, dq $J=11.7$ Hz, $J=4.8$ Hz), 4.52 (1H, d $J=4.2$ Hz), 4.73 (1H, d $J=2.3$ Hz), 7.34 (2H, d $J=8.2$ Hz), 7.85 (2H, d $J=8.2$ Hz), 8.02 ppm (1H, t, NH); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.4$, 28.8, 41.9, 51.5, 52.9, 76.8, 78.7, 126.9, 128.1, 129.1, 144.3, 167.4, 170.7, 172.3 ppm; mp: 215–217 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{18}NO_6$: 320.1129, found 320.1136.

3-Phenethylcarbamoyl-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (72): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.51$ (4H, m), 2.67 (2H, t), 2.82 (2H, s), 3.19 (2H, q), 4.41 (1H, d), 4.72 (1H, d), 7.26–7.17 (5H, m), 7.40 ppm (1H, t NH); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.4$, 28.7, 35.0, 40.12, 51.7, 53.1, 76.8, 78.6, 125.9,128.2, 128.5, 139.5, 170.5, 172.4 ppm; mp: 144–146 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}NO_4$: 290.1387, found 290.1396.

3-(3′-Phenylpropylcarbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (73): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.43$–1–56 (4H, m), 1.64 (2H, quin $J=7.3$ Hz), 2.52(2H, t $J=7.5$ Hz), 2.98 (2H, q $J=6.2$ Hz), 4.47 (1H, d $J=4.3$ Hz), 4.71 (1H, d $J=2.3$ Hz), 7.15–7.28(5H, m), 7.34 ppm (1H, t $J=5.3$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta=28.6$, 28.8, 30.7, 32.5, 38.9, 51.5, 53.0, 76.7, 78.6, 125.6, 128.1, 128.2, 141.7, 170.3, 172.2 ppm; mp: 135–137 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}NO_4$: 304.1543, found 304.1549.

3-(′4-Phenylbutylcarbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (74): ^{1}H NMR ([D$_6$]DMSO): $\delta=1.35$–1.60 (8H, m),

2.51(2H, quin), 2.98 (2H, q), 4.43 91H, d), 4.69 (1H, d), 7.15–7.29 (5H, m), 7.30 ppm (1H, t); ^{13}C NMR ([D_6]DMSO): 28.3, 28.5, 34.7, 38.2, 49.3, 52.3, 53.3, 77.0, 78.5, 125.6, 128.2, 142.1, 170.7, 172.7 ppm; mp: 105–107 °C; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}NO_4$: 318.1700, found 318.1711.

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