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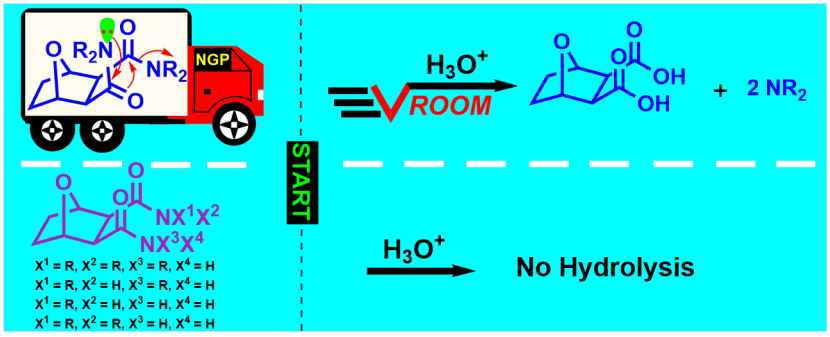

Dual Demeanour of Norcantharidin Derived Dicarboxamides in Acidic Media: An Insight

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Key words: Amide hydrolysis, neighbouring group participation, theoretical calculation, X-ray crystal structure, conformationally constrained diamide, slow drug release

Abstract

The hydrolytic stability of norcantharidine derived conformationally constrained diamides in acidic media is solely governed by the type of amide. Tertiary diamides underwent smooth acid catalyzed hydrolysis due to anchimeric assistance whereas other diamides were stable. This was corroborated from conformational proximity of the amide groups for anchimeric assistance based on single crystal X-ray structure analysis. Theoretical calculations on the diamide structures also predict the similar proximity of the diamides in those conformations. Thus norcantharidine based diamides could probably serve as promising systems for delayed release of certain types of drugs

which possess secondary amine component as exemplified by the release of m-chlorophenylpiperazine at a pH range of 1-2.

Introduction

The amide linkage is a characteristic feature in proteins. Proteinaceous biomolecules like enzymes, hormones etc. are involved in vital functions like catalysis of reactions, transport of molecules and transmission of messages from cell to cell. Amide hydrolysis is often used as a model for the cleavage of peptide bonds.[1,2] Proteolysis catalyzed by proteases regulates several physiological, biochemical and cellular processes. Amide linkage also features in several drugs as well as in a plethora of natural products having a wide range of bioactivities.[3] Owing to this, the amide hydrolysis has been a subject of study by many research groups. Usually amide linkage is chemically robust[4-6] which is due to the resonance between the nitrogen lone pair and the pi-CO bond. Due to the delocalization of the lone pair, amides show a rigid planar conformation with characteristic shortening of C-N bond and elongation of C=O bond.[7] Its non-enzymatic hydrolysis is not an easy task and has to be performed under harsh acidic or basic conditions. Amide activation to perform various synthetic transformations has also been achieved.[8] Lewis acids have been designed to facilitate the alcoholysis and hydrolysis of amides.[9-13] Specific alkyl group on amide structure such as *N*-tropolonyl amides are sensitive to acid catalyzed hydrolysis due to the ease of formation of tropolonium cation.[14] But there are instances when this hydrolysis becomes facile due to structural features of the amides and embedded functional groups. Non-planar deformations activate the amide bond towards the hydrolysis. Twisted amides have been shown to hydrolyze several orders of magnitude faster than their corresponding planar analogs.[15-22] Amide bond cleavage gets accelerated due to 1,3-diaxial interaction with a carboxylic acid in Kemps' acid amides.[23,24] Anchimeric assistance also

known as neighbouring group participation (NGP) by certain functional groups accelerates the amide hydrolysis. The acid catalyzed amide hydrolysis is facilitated by NGP of an alkoxy,[25] a hydroxyl,[26,27] carboxylic acid[28-38] or an amide[39-42] group (Fig. 1). It is a well-known fact that under physiological conditions proteolytic enzymes hydrolyze an amide bond by NGP via a tetrahedral intermediate.

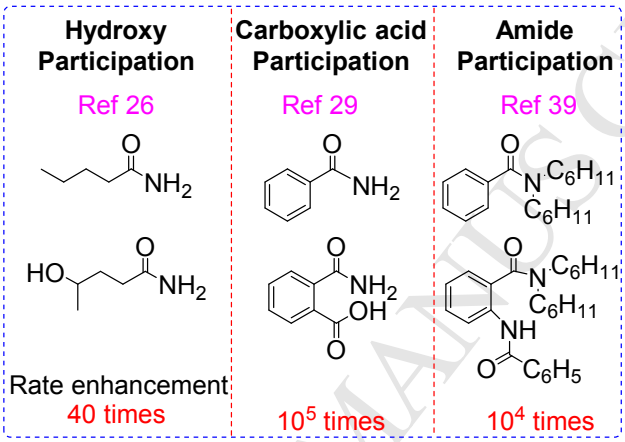


Fig. 1: Rate enhancement of amide hydrolysis due to NGP

Hydroxyl group in γ or δ position of butyramide, valeramide[26] and butyranilide[27] increase the rate constant of acidic hydrolysis. Participation of a carboxylic acid group in the hydrolysis has been reported in conformationally mobile dipeptides,[27] succinanilic acids[31] and aliphatic amide in a cyclohexane system[37] as well as in rigid systems like phthalamic acid derivatives,[29,31] *N*-*o*-carboxybenzoylimidazole,[36] *N*-(*o*-carboxybenzoyl)-L-leucine,[38] 2-acylamidobenzamides,[40] and maleamic acids.[32-35] Amide functionality also aids in the hydrolysis of another proximal amide group as exemplified in *o*-benzamido-*N*,*N*-dicyclohexylbenzamide[39] wherein the hydrolysis occurs at a more bulky amide group. The participation of an amide group is also found in the hydrolysis of *N*-acylimidazole derivatives of *N*-acetylphenylalanine, *N*-

acetylvaline[41] and carbonate diesters.[42] More recently, ammonolysis of a secondary amide assisted by unsubstituted vicinal amide[43] has been reported.

The diamide systems studied so far include either conformationally flexible systems or structurally rigid molecules. None of the previous reports dealt with the hydrolysis of amides in conformationally constrained bridged bicyclic systems with juxtaposed *cis*-dicarboxamides or their closest analogs. Besides, the systems studied never addressed the sensitivity of hydrolysis to the structure of the amine component, i.e. the type of amide. To address these issues, we herein present a systematic study of norcantharidine derived conformationally constrained bridged 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamides (Fig. 2) towards acid catalyzed hydrolysis.

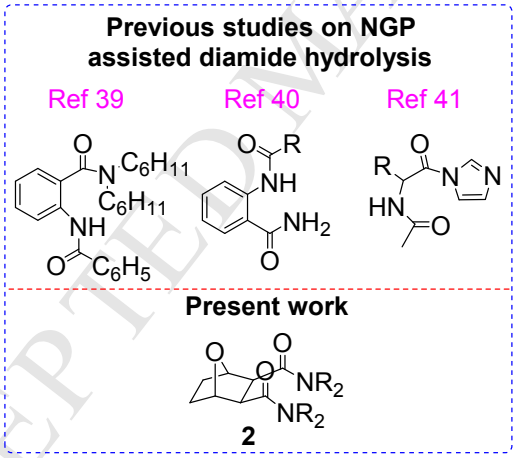


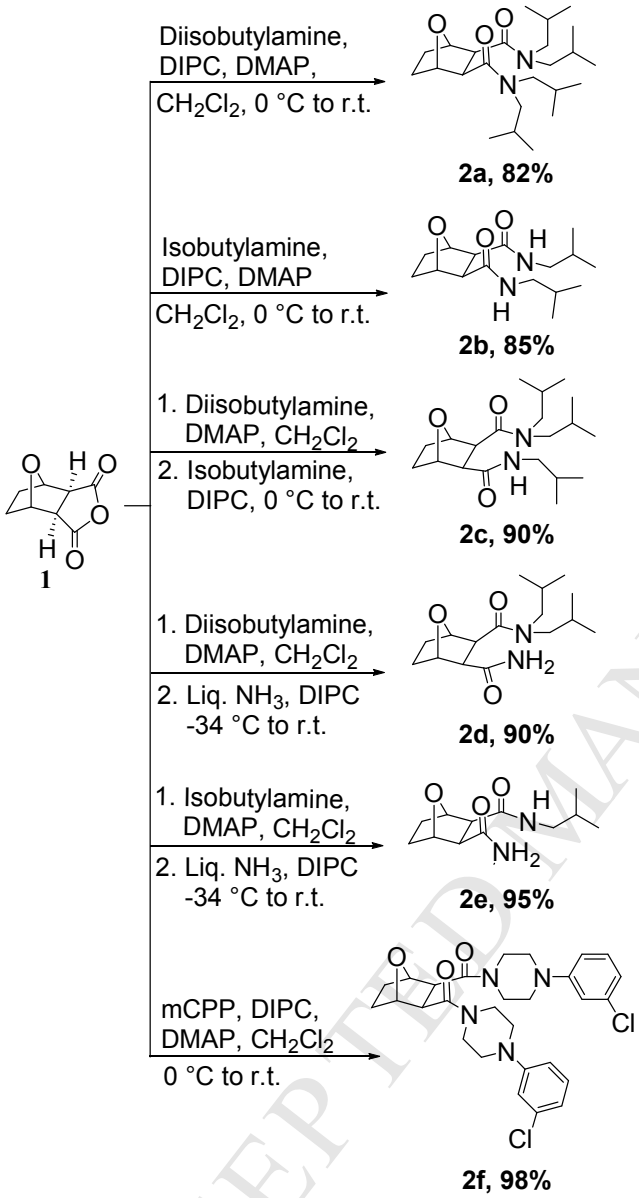
Fig. 2: Hydrolysis studies of diamides

Results and Discussion

During the development of CHON based diamides for nuclear reprocessing,[44] we synthesized conformationally constrained 7-oxabicyclo[2.2.1]heptane2,3-dicarboxamides[45] (Fig. 2) from 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid and long-chain dialkylamines. We experienced a

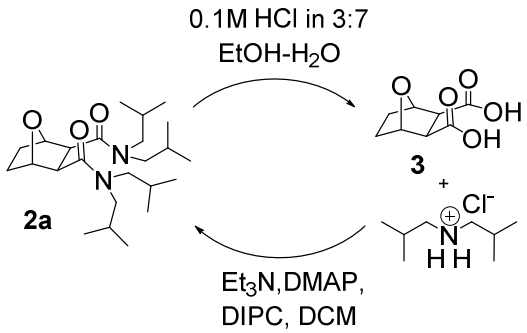
rapid hydrolytic degradation of *N*,*N*,*N*′,*N*′-tetrakis(2-ethylhexyl)-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamide in acidic media. This strange observation prompted us to systematically investigate the hydrolytic behaviour of a series of other 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamides vis-a-vis their conformational preferences.

In order to study the structure-reactivity relationship of the hydrolysis of diamides, dicarboxamides **2a-e** featuring different amine components were synthesized from readily available starting materials as shown in Scheme 1. The reaction of various amines with oxa-bridged tricyclic anhydride **1** in the presence of DMAP and diisopropylcarbodiimde (DIPC) afforded these diamides in good yields. The anhydride **1**, in turn, was prepared by the hydrogenation of the Diels-Alder adduct of furan and maleic anhydride. Amines with shorter alkyl groups were chosen with the anticipation of obtaining good solids and eventually good single crystals. Although the chemical reactivity of both long as well as short carbon chain diamides **2**, would be comparable.



Scheme 1: Synthesis of bridged 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamides.

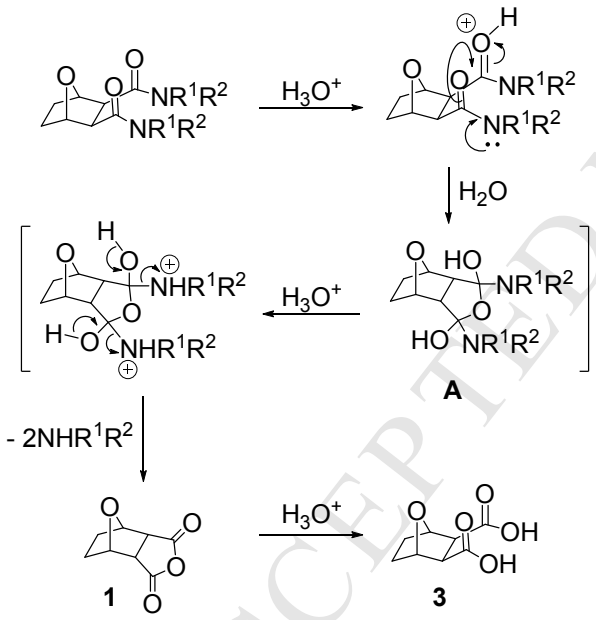
The hydrolysis studies were performed by stirring these diamides in 0.1 M HCl in EtOH/H_2O (30/70) and was monitored by TLC. Under these conditions, diamide **2a** completely hydrolyzed within 0.5 h to produce diacid **3** and diisobutylamine whereas diamides **2b**-**e** did not undergo any hydrolysis (Scheme 2).



Scheme 2: Hydrolysis of **2a**

We were bit surprised to see this unusual hydrolytic behavior reflecting contrasting stability of these diamides **2a-e** in acidic media. The reactivity vs. structural variations in intramolecular systems has been interpreted by several research groups on the basis of orbital steering[46] (angular dependence), proximity,[47] stereopopulation control[48] and spatiotemporal hypothesis.[49] According to Menger's spatiotemporal hypothesis,[49] the type of reaction i.e. intermolecular or intramolecular is governed by the distance between the two reactive centers. When the distance between the two reactive centers exceeds 3 Å, intermolecular reaction is preferred whereas distances below this value allow an intramolecular participation. Further, Karaman[50] expanded and analyzed this study, and combined the hypothesis of all the previous researchers. He postulated that strain energy essentially is a function of distance and angle; both the distance and angle of attack are the governing factors in rate enhancement. In addition to this, the steric dependence on the rate of hydrolytic reactions has also been studied. In case of *o*-benzamido-*N*,*N*-dicyclohexylbenzamide, the hydrolysis occurs at more sterically hindered carbonyl group[30] while an enhancement in rate with every increase in substitution going from succinanilic acid to tetramethylsuccinanilic acid was observed.[39]

The stepwise hydrolysis of diamide **2a** to acid-amide and then to diacid **3** may not be happening as we were unable to trace the formation of monoamide intermediate while monitoring by TLC. The plausible mechanism for the hydrolysis of diamide **2a** has been delineated in Scheme 3. The first step is the protonation of one of the amide carbonyl group. Due to the close proximity, the oxygen of the second carbonyl then attacks the protonated carbonyl group. Subsequent to this in presence of water tetrahedral intermediate "A" is formed. The formation of tetrahedral intermediate[51] was first reported by Bender in the hydrolysis of esters. This intermediate then gets protonated which results in loss of amines and formation of anhydride **1** which eventually in presence of acid and water gets hydrolyzed to the diacid **3**.



Scheme 3: Plausible mechanism for the hydrolysis of diamide **2a**

It was expected that the 3-dimensional structures of these diamides would let us know the proximity of the two carbonyls and their orientation in space shedding light on the reasons behind the quick hydrolysis of diamide **2a** and stability of other diamides **2b-e**. Thus diffraction

quality crystals for these diamides **2a-d**[52] were grown and X-ray crystallographic study was performed.[53] The crystal structures revealed that in all these molecules the two amide substituents are unsymmetrically arranged. The crystallographic asymmetric unit of **2a** has four molecules, whereas **2d** has two molecules and crystallized as a dihydrate. The asymmetric unit of **2b** and **2c** has only one molecule each. In **2a**, the carbonyl oxygen atom of one of the amides lies in close proximity of the carbonyl carbon atom of another amide group. The distance between these carbon and oxygen atoms in **2a** ranges from 2.604-2.676 Å in the four molecules of the asymmetric unit,[54] whereas in case of **2b** and **2c** the corresponding distance (minimum of the two pairs) is 3.058 Å and 3.525 Å, respectively. These distances in **2d** are 3.674 Å and 3.692 Å for the two molecules in the asymmetric unit respectively. In the crystal structure of **2d**, a water molecule bridges the two diamides in the asymmetric unit. It makes a hydrogen bond with the oxygen atoms of the amide from two different diamide molecules directing the crystal packing. Thus the distance between the carbonyl oxygen atom of one of the amide groups and the carbonyl carbon atom of another amide group is relatively small in case of **2a** in comparison to **2b-d** and is below the critical distance of 3 Å for intramolecular participation (Fig. 3).

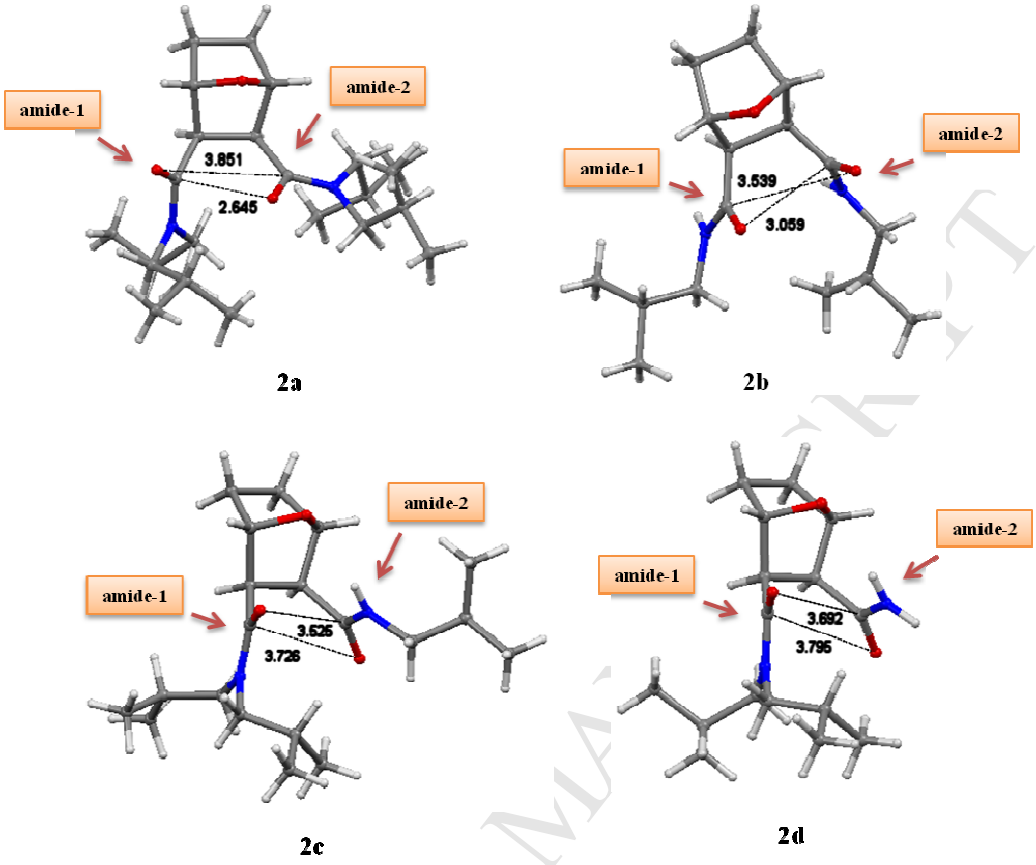


Fig. 3: Single crystal X-ray structures of amides **2a-d**. For simplicity, only one of the molecules present in the asymmetric unit of **2a** and **2d** are shown.[54]

Thus, as the steric crowding in the molecule increases it brings the carbonyl oxygen of one of the amides closer to the carbonyl carbon of another amide, and in case of **2a**, the value was less than 3 Å. This is in accordance with the Menger's postulation[49] on the critical distance of less than 3 Å (diameter of water) to achieve rate acceleration. Also, it has been proposed earlier that the Bürgi–Dunitz angle, [55] O---C(=O) angle, for a successful nucleophilic attack on a carbonyl must be in the range of 105 ± 5°. In the case of **2a**,[54] this angle has been found to be 106.86° (Fig. 4), which lies in this optimum range. In the case of **2b** the angle is 88.4° which is

much less than the optimum for nucleophilic attack. In the case of **2c** and **2d**,[54] the angles are found to be 135.55° and 138.53° respectively, which are larger than the optimum value.

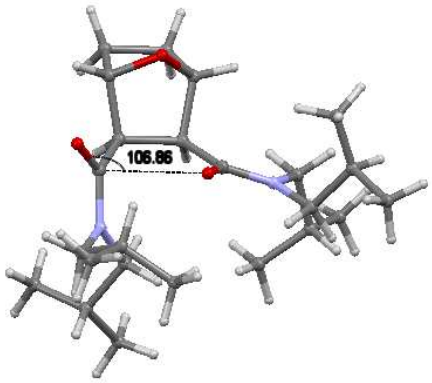


Fig. 4: Bürgi–Dunitz angle for amide **2a**

Another interesting observation was the angle between the planes of two carbonyls. It was found to be about 50° for amides **2b-d** whereas in case of amide **2a** it was nearly 64.0° (Table 1).[54] Due to steric hindrance of the bulky substituents, the diamide **2a** could attain an appropriate conformation, proximity and the angle of attack leading to neighbouring group participation and facilitating rapid hydrolysis in acidic media, whereas other diamides were stable. The twist angle that represents the dihedral angle between C=O and N-R is close to zero for planar amide bond. As the twist angle increases, the amide becomes susceptible for hydrolysis or other nucleophilic attacks. The twist angle in amide-1 for diamides **2b-2d** is lower compared to that of the amide **2a** which is also reflected in the slightly shorter C=O and slightly longer C-N bond in case of **2a** compared to **2b-d** (Table 1). These values, although not very significant, facilitates NGP in **2a** during acid catalysed hdrolysis. In all cases, the distortion angles were found to be <10° which also suggests that individual amide groups are not reactive enough to directly react with water during hydrolysis but are getting hydrolyzed by NGP of C=O

followed by water attack. Although, the conformations adopted by diamides **2a-d** in the solid state might not be the minimum energy conformations (vide infra), they show such conformations may facilitate NGP induced hydrolytic instability.

Table 1: Structural details of diamides **2a-d** from X-ray crystallography[54]

Diamide	Distance (Å) between carbonyl "O" of amide-1 & carbonyl "C" of amide-2	O---C(=O) angle (Bürgi–Dunitz angle)	Amide C=O & C-N bond length (Å) [*cis*-O=C-N-R dihedral angle]	Angle between C=O planes of amide-1 and amide-2
2a	2.645 ± 0.029 & 3.851 ± 0.041	106.86	1.224 & 1.366 [-8.68] (amide-1) 1.247 & 1.348 [0.10] (amide-2)	63.92
2b	3.058 & 3.536	88.40	1.268 & 1.315 [2.47] (amide-1) 1.261 & 1.331 [4.61] (amide-2)	50.57
2c	3.525 & 3.726	135.55	1.231 & 1.349 [0.09] (amide-1) 1.219 & 1.341 [1.56] (amide-2)	51.50
2d	3.692 ± 0.009 & 3.795 ± 0.0055	138.53	1.232 & 1.342 [-0.42] (amide-1) 1.235 & 1.330 [2.06] (amide-2)	49.02

Theoretical calculations were performed using the GAUSSIAN 16 program[56] to examine if the conformations of amides **2a-d** obtained from X-ray crystal structures are the most stable ones and also to predict energy of different possible conformations of these systems. Input structures of different conformations for geometry optimizations were generated considering all possible orientations of the groups attached to the bicyclic bridged moiety. The electronic structure calculations based on first principle have been carried out on systems **2a-d** applying dispersion corrected DFT functional, ωB97X-D in conjunction with 6-311++G(d,p) basis functions. These calculations are carried out in water and ethanol solvents following a macroscopic solvent model SMD, which is known to capture solvent effect reasonably well. In this implicit solvation model, the solute electron density is allowed to interact with the solvent, which is represented as a dielectric continuum. Conformation analyses have been performed on all the four bridged 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamides, **2a-d** keeping bridged oxabicyclic scaffold intact. It is observed that in all the four systems, the conformer that has two carbonyl groups in *anti* position is the most stable one in contrary to the X-ray crystal structure obtained for **2a** and **2b**. In case of **2a**, the most stable conformer (see Fig 5, **2a1**) is calculated to be 3.7 kcal/mol lower in energy than the next stable conformer (see Fig 5, **2a2**) calculated which is similar to the X-ray crystal structure. In case of **2b**, the most stable conformer (see Fig 5, **2b1**) is calculated to be 2.6 kcal/mol lower in energy than the next stable conformer (see Fig 5, **2b2**) calculated which is again similar to the X-ray crystal structure. For, **2c** and **2d** systems, the most stable structures are similar to the X-ray crystal structure. It may be noted from Fig. 5 that for **2a2**, the inter-atomic distance between carbonyl oxygen on one amide and carbonyl carbon of other amide is suitable for NGP as the calculated shortest distance is 2.7 Å. In case of **2b2**, the said distance is 2.96 Å and it is expected to be suitable for NGP as per Menger postulate.[49]

However, no reaction took place in case of **2b2** suggesting the distance of 2.96 Å is not close enough to accelerate NGP induced hydrolysis. The ΔE in the figure shows the relative energy with respect to the most stable conformer of **2a** and **2b** i.e. **2a1** and **2b1**, respectively. The calculated ΔE for **2a2** and **2b2** indicates the strain energy of the two systems with respect to the most stable conformers. Similar calculations on diamide **2c** showed that the *syn* carbonyls conformer is 2.4 kcal/mol higher in energy than that of the *anti* i.e. the stable conformer while in compound **2d**, the stable *anti* carbonyls conformer is 1.8 kcal/mol lower in energy than that of the *syn* carbonyls conformer. Also, the minimum distance between the two amides in *syn* carbonyls conformer of **2c** and **2d** was calculated to be 2.92 Å and 2.97 Å, respectively which are not favorable for NGP. Single crystal X-ray crystallography suggests the presence of a water molecule with the diamide in case of structure **2d**. However, present calculations in presence and absence of an explicit solvent water molecule do not indicate any extra stability due to H-bonding interaction with the solvent water molecule.

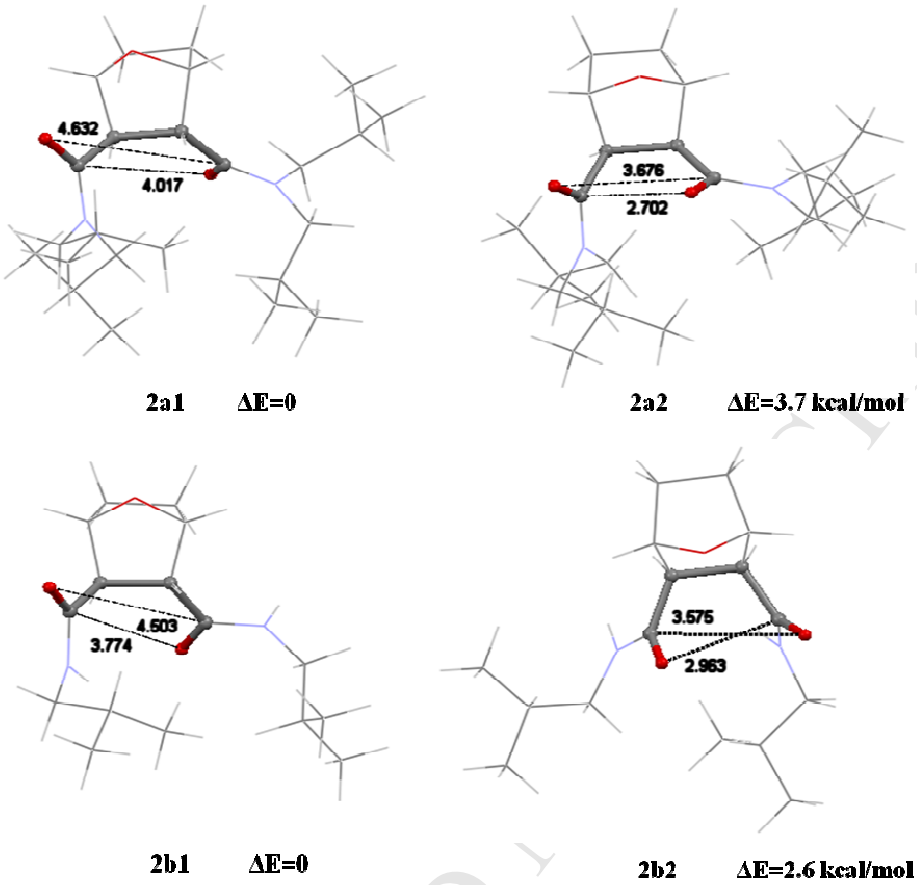


Fig. 5: Calculated conformers of amides **2a** and **2b** with *syn* and *anti* C=O groups of two amides

We perceived that the exceptional property of the tertiary diamides of type **2a** could probably be employed for delayed release[57] of certain drugs. To exemplify this, anhydride **1** was reacted with *m*-chlorophenylpiperazine (mCPP, an antidepressant metabolite of Trazodone) in the presence of DMAP, DIPC and Et_3N to obtain the diamide **2f** (Scheme 1). This was then subjected to acidic hydrolysis in 0.1 M and 0.01 M HCl in EtOH/H_2O (9:1) whereby mCPP got completely released in a span of about 0.5 h and 13 h, respectively.

Conclusions

In conclusion, the structural and functional requirements for acid catalyzed hydrolytic stability of conformationally constrained bridged 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxamides were derived in combination with hydrolytic studies, X-ray structures and theoretical calculations. The hydrolysis of tertiary-tertiary diamides was attributed to the NGP of the amide groups which are in close proximity and correct orientation. Except for tertiary-tertiary diamides, rest all types were stable under acidic hydrolytic conditions. Owing to this dual property, these diamides **2** hold promises for multiple uses including pH dependent sensor, metal extraction from acidic waste and slow release of certain drugs.

Experimental Section

General Methods. All reactions were performed in oven-dried (120 °C) or flame-dried glass apparatus under dry N_2 or argon atmosphere. Diisobutylamine and isobutylamine were obtained from Aldrich and distilled over CaH_2 prior to use. DIPC was freshly distilled before use. Other chemicals were used as obtained from Aldrich. TLC was carried out using Merck silica gel 60 F_{254} plates. Column chromatography was performed on silica gel (230–400 mesh). ^{1}H NMR spectra were recorded on 200, 500 and 600 MHz spectrometers and $^{13}C\{^{1}H\}$ NMR spectra were recorded with 50 and 125 MHz spectrometers using $CDCl_3$ as the solvent. The spectra were referenced to residual chloroform (δ 7.25 ppm, ^{1}H; 77.00 ppm, ^{13}C). IR spectra were recorded on the FT-IR spectrometer using NaCl discs, and wavenumbers are given in cm $^{-1}$. Melting points (mp) were uncorrected. Elemental analyses (C, H, N) were carried out by Elementar, vario MICRO CHNS instrument. HR-MS spectra were obtained using a high-resolution ESI-TOF mass spectrometer.

Computational method. The electronic structure calculations are carried out applying density functional theory (DFT) based method. As the systems are expected to have non-bonding interactions, a DFT functional including empirical dispersion correction, namely, ωB97X-D is considered for present calculations. This DFT functional is known to perform well for such systems. A large set of atomic basis function, 6-311++G(d,p) is adopted for all calculations to account non-bonding interactions accurately. All calculations are carried out in water as well as in ethanol medium applying SMD macroscopic solvent model. Input geometries are considered based on X-ray structures as well as all structures possible from different orientations of groups attached to the rigid bicyclic bridged moiety. Geometry optimizations for all the input guess structures are calculated following Newton-Raphson algorithm. Hessian calculations are carried out for all the predicted equilibrium structures and it is observed that these are true minima. These calculations are performed using GAUSSIAN 16 Revision A.03 suit of *ab initio* program.

X-ray crystallographic method. Single crystal X-ray diffraction data were collected on Agilent Supernova system equipped with a microfocus Cu-source (λ = 1.5418 Å) and a Titan CCD detector. The crystals were separated, coated with paraffin oil and mounted on a loop for X-ray diffraction data collection at specified temperature. The data reduction and analysis were carried out with CrysAlisPro software suit. Analytical absorption correction using a multifaceted crystal model based on expressions derived by Clark & Reid[58] and as implemented in the CrysAlisPro software suit was carried out for both the crystals. The structures were solved by direct method using Shelxs and refined using Shelxl softwares[59] using Olex2 interface.[60] All the non-hydrogen atoms were refined anisotropically and hydrogens were generated at their idealized positions and refined isotropically according to riding model.

Synthetic procedure and analytical data of 2a-f

N^2,N^2,N^3,N^3-tetraisobutyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxamide (2a). A solution of diisobutylamine (1.4 mL, 8 mmol) in dry CH_2Cl_2 (2 mL) was added to a stirred solution of anhydride **1** (672 mg, 4 mmol) and DMAP (98 mg, 0.8 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. After 0.5 h, the reaction mixture was cooled on ice-water bath and DIPC (0.6 mL, 4 mmol) was added drop-wise into it. The reaction mixture was stirred overnight and allowed to attain room temperature. The reaction mixture was diluted with 1/1 ethyl acetate-petroleum ether and washed with a dilute citric acid solution, several times with water to remove DMAP and diisopropylurea. The organic extract was concentrated under reduced pressure and the residue was purified by column chromatography to give **2a**[45] (1.35 g, 82%) as a white solid. The product was crystallized from ethyl acetate/petroleum ether to obtain suitable crystals for single-crystal X-ray analysis. mp 144-146 °C; IR (film) *ν*max = 2959, 2872, 1633, 1454, 1152 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.89 (s, 2 H), 3.14 (dd, *J* = 13.5, 8.0 Hz, 2 H), 3.07 (dd, *J* = 13.5,7.0 Hz, 2 H), 3.00-2.91 (m, 6 H), 2.12 (nonet, *J* = 6.5 Hz, 2 H), 1.94 (nonet, *J* = 6.5 Hz, 2 H), 1.86-1.84 (m, 2 H), 1.56-1.53 (m, 2 H), 0.92 (d, *J* = 6.5 Hz,12 H), 0.88 (d, *J* = 6.5 Hz, 6 H), 0.85 (d, *J* = 6.5 Hz, 6 H) ppm; $^{13}C\{1H\}$ NMR (125 MHz, $CDCl_3$) δ 170.0 (2C), 79.6 (2C), 55.7, 54.0, 51.5 (2C), 29.7 (2C), 27.8 (2C), 26.4 (2C), 20.7 (2C), 20.6 (4C), 20.4 (2C), 20.1 (2C) ppm; Anal. Calcd for $C_{24}H_{44}N_2O_3$: C, 70.5; H, 10.85; N, 6.9. Found: C, 70.2; H, 10.6; N, 7.2.

N^2,N^3-diisobutyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxamide (2b). A solution of isobutylamine (6 mmol) in dry CH_2Cl_2 (1 mL) was added to a solution of anhydride **1** (504 mg, 3 mmol) and DMAP (73 mg, 0.6 mmol) in dry CH_2Cl_2 (9 mL) at room temperature. The reaction mixture was stirred and cooled on ice water bath. DIPC (0.7 mL, 4.5 mmol) was added drop-wise to stirred reaction mixture. The reaction mixture was allowed to attain room temperature

and stirred overnight. The reaction mixture was diluted with 1/1 ethyl acetate-petroleum ether and washed with dilute citric acid solution, several times with water to remove DMAP and diisopropylurea. The organic extract was concentrated under reduced pressure and the residue was purified by column chromatography to give **2b** (760 mg, 85%) as a white solid. The product was crystallized from ethyl acetate/petroleum ether to obtain suitable crystals for single-crystal X-ray analysis. mp 224-225 °C; IR (film) *v*max = 3332, 3293, 2955, 1655, 1649, 1215 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.08 (s, broad, 2 H), 4.94 (bd, *J* = 2 Hz, 2 H), 3.01-2.92 (m, 4 H), 2.92 (s, 2 H), 1.86-1.78 (m, 2 H), 1.70 (nonet, *J* = 6.5 Hz, 2 H), 1.55-1.48 (m, 2 H), 0.89 (d, *J* = 6.5 Hz, 12 H) ppm.$^{13}C\{1H\}$ NMR (125 MHz, $CDCl_3$) δ 171.0 (2C), 78.5 (2C), 54.7 (2C), 47.0 (2C), 28.8 (2C), 28.3 (2C), 20.1 (4C) ppm; Anal. Calcd for $C_{16}H_{28}N_2O_3$: C, 64.8; H, 9.5; N, 9.45. Found: C, 64.9; H, 9.3; N, 9.8.

N^2,N^2,N^3-triisobutyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxamide (2c). A solution of diisobutylamine (4 mmol) in dry CH_2Cl_2 (1 mL) was added to a solution of anhydride **1** (672 mg, 4 mmol) DMAP (49 mg, 0.4 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. After overnight stirring, the reaction mixture was cooled on an ice-water bath and a solution of isobutylamine (4 mmol) and DIPC (0.6 mL, 4 mmol) in dry CH_2Cl_2 (1 mL) was added to reaction mixture. After stirring for 5 h, the reaction mixture was diluted with 1/1 ethyl acetate-petroleum ether and washed with dilute citric acid solution, several times with water to remove DMAP and diisopropylurea. The organic extract was concentrated under reduced pressure and the residue was purified by column chromatography to give **2c** (1.26 g, 90%) as a white solid. The product was crystallized from ethyl acetate/petroleum ether to obtain suitable crystals for single-crystal X-ray analysis. mp 122-124 °C; IR (film) *v*max = 3383, 2962, 2931, 1656, 1633, 1215 cm^{-1}; ^{1}H NMR (500 MHz,$CDCl_3$) δ 6.61 (bd, *J* = 4.0 Hz, 1 H), 5.06 (d, *J* = 4.5 Hz, 1 H), 4.64 (d, *J* = 5.0

Hz,1 H), 3.64 (dd, *J* =13.5, 6.0 Hz, 1 H), 3.31 (dd, *J* = 14.5, 8 Hz, 1 H), 3.29 (t, *J* = 7.5 Hz, 1 H), 3.02 & 3.00 (ABq, *J* = 10.0 Hz, 2 H), 2.91 (dd, *J* = 15.5, 6.0 Hz, 1 H), 2.57-2.50 (m, 1 H), 2.48 (dd, *J* = 13.5, 8.5 Hz, 1 H), 1.96-1.81 (m, 3 H), 1.77 (tt, *J* = 11.5, 4 Hz, 1 H), 1.71 (nonet, *J* = 6.5 Hz, 1 H), 1.55 (td, *J* = 11.5, 3.5 Hz, 1 H), 1.49 (dq, *J* = 11.0, 3 Hz, 1 H), 0.95 (d, *J* = 6.5 Hz, 3 H), 0.91-0.85 (m, 12 H), 0.82 (d, *J* = 7.0 Hz, 3 H) ppm; ^{13}C{1H} NMR (125 MHz, $CDCl_3$) δ 170.7, 170.4, 79.8, 78.3, 56.9, 55.7, 54.0, 50.8, 46.4, 29.5, 28.5, 28.4, 27.5, 26.5, 20.7, 20.5, 20.4, 20.1, 20.0, 19.9; Anal. Calcd for $C_{20}H_{36}N_2O_3$: C, 68.1; H, 10.3; N, 7.95. Found: C, 68.1; H, 10.5; N, 7.7.

***N*^{2},*N*^{2}-diisobutyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxamide (2d)**. A solution of diisobutylamine (0.5 mL, 3 mmol) in dry CH_2Cl_2 (5 mL) was added to a solution of anhydride **1** (500 mg, 3 mmol) and DMAP (80 mg, 0.6 mmol) in dry CH_2Cl_2 (5 mL) at room temperature. After stirring the reaction mixture for about 1 h, about 20 mL of liquid ammonia was collected into the reaction mixture followed by addition of DIPC (0.465 mL, 3 mmol). The reaction mixture was then allowed to attain room temperature gradually over 4 h. The reaction mixture was diluted with chloroform, washed with dilute citric acid solution and several times with water to remove DMAP and diisopropylurea. The organic layer was concentrated under reduced pressure. The residue was purified by column chromatography to give **2d** (795 mg, 90%) as a white solid. The product was crystallized from ethyl acetate/petroleum ether to obtain suitable crystals for single-crystal X-ray analysis. mp 130-131.7 °C; IR (film) *ν*max = 3441, 3501, 2957, 2872, 1676, 1631, 1554, 1466, 1431, 1387, 1236, 1148, 996 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.51 (s, 1 H), 5.10 (d, *J* = 4.8 Hz, 1 H), 5.03 (s, 1 H), 4.70 (d, *J* = 5.4 Hz, 1 H), 3.73 (dd, *J* = 13.2, 6.0 Hz, 1 H), 3.27 (dd, *J* = 15.0, 9.6 Hz, 1 H), 3.03 (d, *J* = 9.6 Hz, 1 H), 2.97 (d, *J* = 9.6 Hz, 1 H), 2.94 (dd, *J* = 15.6, 6.0 Hz, 1 H), 2.46 (dd, *J* = 13.2, 7.8 Hz, 1 H), 2.00-1.88 (m, 2 H), 1.86

(ddd, *J* = 12.0, 4.8, 3.6 Hz, 1 H), 1.80 (tt, *J* = 12.6, 4.8 Hz, 1 H), 1.56 (td, *J* = 12.0, 4.2 Hz, 1 H), 1.49 (td, *J* = 10.2, 3.6 Hz, 1 H), 0.95 (d, *J* = 6.6 Hz, 3 H), 0.89 (d, *J* = 6.6 Hz, 3 H), 0.87 (d, *J* = 7.2 Hz, 3 H), 0.83 (d, *J* = 6.6 Hz, 3 H) ppm; ^{13}C{1H} NMR (50 MHz, $CDCl_3$) δ 173.1, 170.2, 79.1, 78.0, 56.4, 55.3, 53.4, 50.1, 29.1, 28.4, 27.1, 26.1, 20.3, 20.2, 20.1, 19.5 ppm; HRMS (ESI-TOF) *m/z* $[M + Na]^+C_{16}H_{28}N_2NaO_3$ calcd for 319.1992, found 319.1992.

N*^{2}*-isobutyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxamide (2e). A solution of isobutylamine (0.3 mL, 3 mmol) in dry CH_2Cl_2 (5 mL) was added to a stirred solution of anhydride **1** (500 mg, 3 mmol) and DMAP (80 mg, 0.6 mmol) in dry CH_2Cl_2 (5 mL) at room temperature. After stirring the reaction mixture for about 1 h, about 20 mL of liquid ammonia was collected into the reaction mixture followed by addition of DIPC (0.465 mL, 3 mmol). The reaction mixture was then allowed to attain room temperature gradually over 4 h. The reaction mixture was diluted with chloroform, washed with citric acid solution and several times with water to remove DMAP and diisopropylurea. The organic layer was concentrated under reduced pressure. The residue was purified by column chromatography to give **2e** (680 mg, 94%) as a white solid. mp 222.3-223.2 °C; IR (film) *ν*max = 3289, 3336, 2956, 2870, 1650, 1554, 1463, 1385, 1242, 1168, 936, 816 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.29 (s, 1 H), 6.14 (s, 1 H), 5.32 (s, 1 H), 4.97 (br t, *J* = 2.6 Hz, 1 H), 4.89 (br t, *J* = 2.8 Hz, 1 H), 3.02 (dd, *J* = 6.2, 3.0 Hz, 1 H), 2.98 (dd, *J* = 6.2, 2.8 Hz, 1 H), 2.95 (s, 2 H), 1.90-1.60 (m, 3 H), 1.60-1.45 (m, 2 H), 0.90 (s, 3 H), 0.87 (s, 3 H) ppm; ^{13}C{1H} NMR (125 MHz, $CDCl_3$) δ 172.8, 171.3, 78.8, 78.1, 54.8, 54.3, 47.1, 28.9, 28.8, 28.3, 20.1, 20.1 ppm; HRMS (ESI-TOF) *m/z* $[M + Na]^+C_{12}H_{20}N_2NaO_3$ calcd for 263.1366, found 263.1364.

7-Oxabicyclo[2.2.1]heptane-2,3-diyl)bis((4-(3-chlorophenyl)piperazin-1-yl)methanone (2f). Triethylamine (0.84 mL, 6 mmol) was added to a suspension of anhydride **1** (504 mg, 3 mmol),

m-chlorophenylpiperazine (1.4 g, 6 mmol) and DMAP (73 mg, 0.6 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was cooled on ice bath and DIPC (0.5 mL, 3 mmol) was added drop-wise to it. The reaction mixture was stirred overnight and allowed to attain room temperature. The reaction mixture was diluted with 1/1 ethyl acetate-petroleum ether and washed with dilute citric acid solution, several times with water to remove DMAP and diisopropylurea. The organic extract was concentrated under reduced pressure and the residue was purified by column chromatography to give **2f** (1.57 g, 98%) as a white solid. mp 195.5-196.5 °C; IR (film) *ν*max = 2827, 1649, 1593, 1485, 1432, 1230, 1103, 990 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (t, *J* = 8 Hz, 2 H), 6.84 (br s, 4 H), 6.75 (d, *J* = 8 Hz, 2 H), 4.93 (s, 2 H), 3.90-3.80 (br m, 2 H), 3.70-3.55 (br m, 4 H), 3.55-3.45 (m, 2 H), 3.30-3.00 (m, 10 H), 1.87 (d, *J* = 8 Hz, 2 H), 1.59 (d, *J* = 7.5 Hz, 2 H) ppm; ^{13}C{1H} NMR (125 MHz, $CDCl_3$): δ 169.2, 151.9, 135.0, 130.2, 120.0, 116.2, 114.3, 78.8, 51.8, 48.9, 48.7, 45.2, 41.8, 29.5 ppm; Anal. Calcd for $C_{28}H_{32}Cl_2N_4O_3$: C, 61.9; H, 5.9; N, 10.3. Found: C, 62.1; H, 5.55; N, 10.1.

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Appendix A. Supplementary data

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

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52. Crystallographic data for diamide derivatives **2a-d** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos. CCDC 1888170 (**2a**), 1888171 (**2b**), 1888168 (**2c**) and 1888169 (**2d**).

53. The X-ray crystal structure revealed that in the asymmetric unit, four **2a** molecules are present and two **2d** molecules are present; For details, see SI.

54. The relevant data for other structures in the asymmetric unit of **2a** and **2d** are provided in the SI.

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Highlights

Norcantharidine derived tertiary diamides hydrolysed easily under acid catalysis

Their primary, secondary and mixed diamides do not hydrolyse easily

Hydrolysis of tertiary diamides occurred due to NGP of proximal amide carbonyls

Hydrolysis of tertiary diamides has been supported by X-ray structure & calculations

These tertiary diamides could serve for delayed release of certain types of drugs