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The Versatility of Squaramides: From Supramolecular Chemistry to Chemical Biology

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Abstract- This review covers recent advances in the use of the squaramide moiety in chemical research. We focus on the varied applications of squaramides under the broad headings of self-assembly, organocatalysis, molecular recognition, medicinal chemistry, and bioconjugation and highlight several examples of each application.

1. INTRODUCTION

Squaramides, a family of conformationally rigid cyclobutene ring derivatives, are rapidly gaining research interest across diverse areas of the chemical and biological sciences.1–3 Composed of two carbonyl hydrogen-bond acceptors in close proximity to two NH hydrogen-bond donors, this small molecular scaffold benefits from unique physical and chemical properties that render it extremely useful as a tool in areas as diverse as catalysis, molecular recognition, bioconjugation, and self-assembly. One of its most striking properties arises from the delocalization of a nitrogen lone pair into the cyclo-butenedione ring system conferring the four-membered ring with aromatic character (Hu¨ ckel's rule: [4n + 2] p electrons, n = 0). In addition, the capacity of squaramides to form strong hydrogen bonds that simultaneously increase the aromatic character of the four-membered ring is highly advantageous where self-assembly and molecular recognition processes can benefit from favorable thermodynamic stability brought about by aromatic gain.4,5 This fact, along with synthetic versatility, conformational rigidity, and relative stability, has stimulated a burgeoning research effort over the past number of years toward exploiting this most useful of scaffolds. Derived from squaric acid (diketocyclobutenediol), itself first synthesized by Cohen et al. in 1959 via the

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hydrolysis of dichlorotetrafluorocyclobutene,6 it was West and colleagues who provided an explanation for the stability and aromaticity of the dianionic diketocyclobutene.7 Although a large body of work on squarate derivatives has been theoretical in nature, establishing the relative aromaticity of CnOn 2 systems,8,9 it is the facile synthesis of alkyl squarates that has opened the door to the synthetic accessibility of the squaramide that we enjoy today.10 One of their particularly useful characteristics is the ability to be sequentially substituted whereby increased aromatic stabilization afforded by the first substitution reaction relative to the parent alkyl squarate is thought to render the monosubstituted intermediate less reactive thus allowing facile synthetic access to unsymmetrical squaramides.9 Indeed, the alkyl esters have become increasingly useful tools as bioconjugation moieties and as the key starting materials for the synthesis of both mono- and di-substituted squaramides11 and squaraine dyes12 and most recently for the facile synthesis of thiosquaramides.13 With the increasing research interest in exploiting the squaramide moiety, the aim of this review article is to focus on some recent advances in the field and highlight some of the interdisciplinary applications of the squaramides. We will focus on the use of squaramides at the borders of materials.

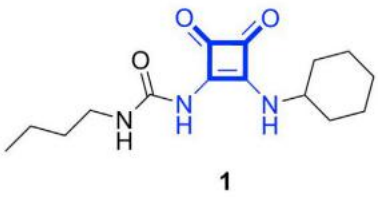

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Figure 1. Chemical Structure of Compound 1 and Its X-Ray Crystal Structure Showing the Relationship between Adjacent Hydrogen-Bonded Molecules

science and biology under the broad headings of self-assembly, organocatalysis, molecular recognition, medicinal chemistry, and bioconjugation, where a number of recent elegant examples have been reported. Although non-exhaustive, this review aims

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to include a selection of recently published examples to highlight the utility, diversity, and future potential that this most useful of scaffolds holds.

SQUARAMIDES AS SUPRAMOLECULAR SELF-ASSEMBLY MOTIFS

Molecular self-assembly has emerged as a particularly useful approach for the bottom-up assembly of nanoscale materials where a wide variety of morphologies can be achieved either in bulk or in solution ranging from cylinders and spheres to micelles and vesicles.14,15 The shape and size of self-organized morphologies can be accurately controlled both through judicious design and through a number of variables such as concentration, light, pH, temperature, etc.16 As mentioned above, squaramides benefit from several characteristics that make them amenable for use in self-assembled materials, in particular their structural rigidity, aromaticity, and ability to form strong two-dimensional hydrogen bonds. This has led to a number of reports detailing the formation of non-covalent bonding networks, soft materials, and metal-organic frameworks.

One of the earliest examples of supramolecular self-assembly based on the squaramide motif was reported by Davis and co-workers who described the synthesis of compound 1 (Figure 1).17 X-ray crystallography analysis of 1 revealed that the carbamoyl squaramide unit displayed planarity around the four-membered ring but importantly showed intramolecular hydrogen bonding. 1 was shown to exist as a centrosymmetric dimer in the solid state, sustained by four NH.O=C hydrogen bonds. The structure of the 1:1 dimer was confirmed by calculation of dimerization constants (Kd) (values range from 5 3 103 to 104 M–1 with chemical shift differences ddimer – dmonomer y 2.3–3 ppm) with 1 H NMR dilution analysis in CDCl3–CD3CN (95:5). Costa et al. subsequently reported further conformational analysis of secondary squaramide models 2–5 through NMR analysis in a range of different solvent systems (Figure 2).18 The C–N bonds of several squaramide compounds were shown to be analogous to amide like structures whereby partially restricted rotation around the C–N bond gave rise to syn- and anti-conformations. The study showed that an energy barrier z 63 kJ Mol–1 exists between the syn- and anti-modes of the various

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squaramide structures, allowing ready interconversion between the two conformers at room temperature (RT). In addition, mixtures of syn/anti- and anti/anti-conformations could be observed through molecular mechanics calculations and X-ray crystallographic analysis, thus supporting the earlier NMR evidence; however, the

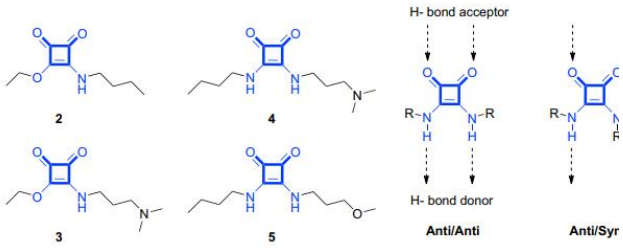

Figure 2. Chemical Structures of Compound 2–5 and Representations of the anti/anti- and anti/ syn-Conformations of Bis-secondary Squaramides

Prohens and co-workers have conducted several follow up conformational studies on a range of squaramides under variable conditions. In one report, three polymorphs of dibenzylsquaramide 6 were examined in different solvents and at varying temperatures (Figure 3).19 Synthesized through reaction between diethylsquarate and benzylamine in ethanol, 6 was subjected to polymorphic screening in different combinations of DMF and DMSO with polar and non-polar solvents.

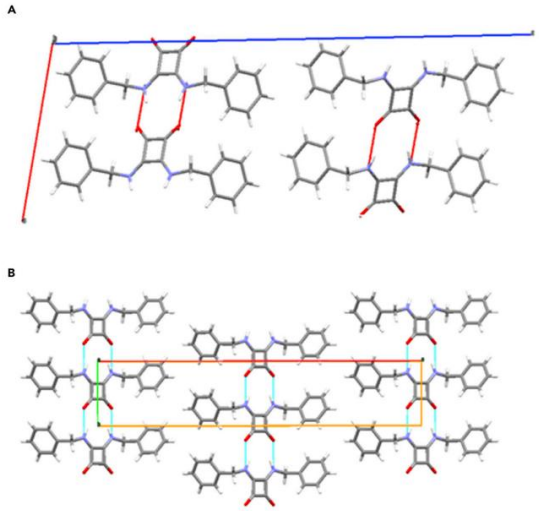

Figure 3. Single-Crystal X-Ray Structures of Forms 6A and 6C

DSC analysis revealed three distinct polymorphs and showed that forms 6B and 6C transform into form 6A at 257C. Crystal structures of metastable forms 6A and 6C were studied by single-crystal X-ray diffraction (XRD) and demonstrated welldefined head-to-tail hydrogen bonding (N–H$$$O 2.834 and 2.779 A˚ , respectively) between the intermolecular squaramide units with different packing directions. Form 6A showed parallel packing but formed an antiparallel chain, whereas form 6C exhibited an anti-parallel packing arrangement with parallel chains. This study confirmed earlier reports18 of the favorable anti/anti-conformation in the solid structure of simple squaramides (Figure 3). Moreover, the inability to distinguish

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between these three polymorphs by differential scanning calorimetry (DSC) told a cautionary tale to those involved in pharmaceutical research about the limitations of DSC when characterizing crystalline solids. A follow-up study examined cooperativity in solidstate squaramides.20 Here, polymorph preferences of a dipyridyl squaramide 7 were examined, and conformational equilibrium constants were calculated at different concentrations of CHCl3. The results showed the existence of two dimers in solution, forms 7A and 7B (Figure 4A), and revealed that form 7B dominates in supersaturated CHCl3 solution.

The authors also performed a polymorphic screening under varying thermodynamic and kinetic conditions. Just two polymorphic forms, forms 7I and 7II, directly related to the form 7A dimer (head-to-tail motif) were obtained, and there was no evidence of the existence of form 7B. However, DSC and thermomicroscopy analysis suggested an enantiotropic relationship between the two polymorphs whereby form 7I (melting point [m.p.] = 166C) showed more stability below the transition temperature (

Prohens, Portell, and Alcobe´ later investigated the preorganization effect on the polymorphism and co-crystallization of squaramide 8 and described new chain and ribbon synthons (Figure 5).21 Bis-squaramide 8 with four predicted supramolecular synthons indeed produced four different solid-state structures through varying intramolecular hydrogen bonding. The structures of the different synthons were characterized by powder XRD, where two of the synthons showed a trans-configuration (a head-to-tail polymer and an intramolecular monomer) and the remaining two synthons showed a cis-configuration (a ribbon assembly and another intramolecular monomer). The head-to-tail polymer (form 8A) was obtained in pure form during the synthesis, whereas the ribbon assembly (form 8B) was obtained through slow-rate crystallization after form 8A melted at 160C. As expected, form 8A presented the, by now well-known, head to-tail hydrogen-bonding motif associated with the squaramide structure and additionally demonstrated p-stacking interactions that resulted in the parallel layers observed in the polymorphic structure (dcentroids 3.851 A˚). Form 8B contained a pseudo six-membered ring structure perpendicular to the plane defined by the piperazine ring again brought about by the propensity of the squaramide to

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partake in intramolecular hydrogen bonding between the amidic NH and the piperazine nitrogen [NH$$$N 2.843(8) A˚] and resulted in a symmetric shortening of the assembly.

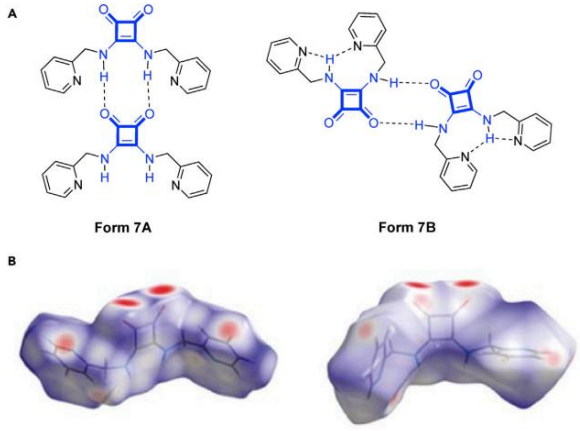

Figure 4. Polymorphic Behaviour of Dipyridyl Squaramide 7

Portel and Roffel continued to pursue the synthesis of supramolecular synthons based on the squaramide motif and reported the helical crystal structure of a disquaramide compound 9 through powder XRD (Figure 6).22 The asymmetric compound 9 was synthesized by a condensation reaction between diethylsquarate and N,N-dimethylethylenediamine and tyramine. The result was an achiral compound capable of forming both clockwise and anti-clockwise rotating helical assemblies in a racemic crystal structure. Again, the self-assembly of 9 exhibited strong CO$$$HN hydrogen bonding within the squaramide portion of the molecule (the aforementioned head-to-tail motif) and provided a skeletal template for peripheral interactions to occur between a tertiary amine and a phenolic hydroxyl group. Additional weak CH$$$p interactions between the methylamino group and the aromatic phenol ring were also thought to contribute to the helical packing.

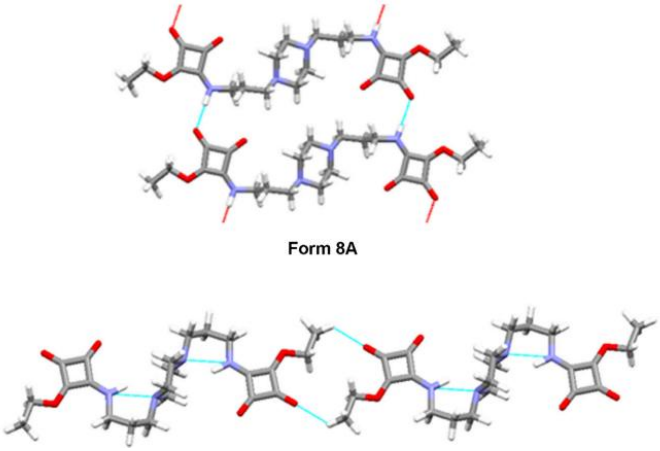

Figure 5. XRD Structure of Head-to-Tail Polymer Form 8A and Ribbon Assembly Form 8B

More recently, Portell, Bardia, and Prohens reported another supramolecular assembly based on the squaramide scaffold, this time with zwitterionic squaramide compound 10. 23 The synthesis of 10 was achieved in a single step from squaric acid and N,N-dimethylethylendiamine in water and was

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expected to yield two distinct supramolecular synthons through charge-assisted hydrogen-bond formation and face-to-face p-stacking (Figure 7A). Indeed, through a polymorph screening, the structures of two anhydrate polymorphs (forms 10I and 10II) and a hydrate (form 10III) were solved by single-crystal XRD. Both anhydrous crystals showed similar NH$$$O interactions between adjacent dimers but with different centroids distances (3.47 and 3.70 A˚ , respectively). The most important difference between forms 10I and 10II stems from the carbonylic oxygen involved in intermolecular hydrogen bonding. Whereas in form 10I the oxygen is syn with respect to the NH.

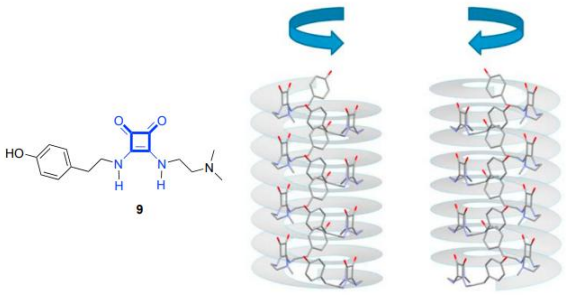

Figure 6. Chemical Structure of Compound 9 and the Resulting Clockwise and Anti-clockwise Rotating Supramolecular Helical Assemblies.

with which it is forming the hydrogen bond, in Form 10II this interaction is anti. This minor difference in conformation leads to important consequences for the connection of the electrostatically compressed dimers, giving rise to completely different supramolecular synthons for both polymorphs: form I yields chains, whereas form II yields rings (Figure 7B). Interestingly, a dissimilar supramolecular synthon is obtained in the hydrate form 10III, where the addition of water molecules results in a stabilization of the entire structure through hydrogen-bonding interactions between the water molecules and the free carbonyls of a neighboring dimer. Self-assembly of squaramides promoted by hydrogen-bonding interactions with a range of anions has also garnered significant research interest. One of the examples reported by Costa and co-workers in 2011 provided a combined crystallographic and computational study concerning the first example of a squaramide-nitrate salt crystal structure.24 The study reported the synthesis of compound 11 and its subsequent crystallization from EtOH solution (Figure 8). The X-ray structure of 11 exhibited the familiar intermolecular head-to-tail arrangement by the formation of N–H$$$O hydrogen bonds between neighboring

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squaramide groups. The hydrogen-bonded network revealed additional stability promoted by the syn orientation of both the pyridine N atom and two additional C–H$$$N bonding interactions.

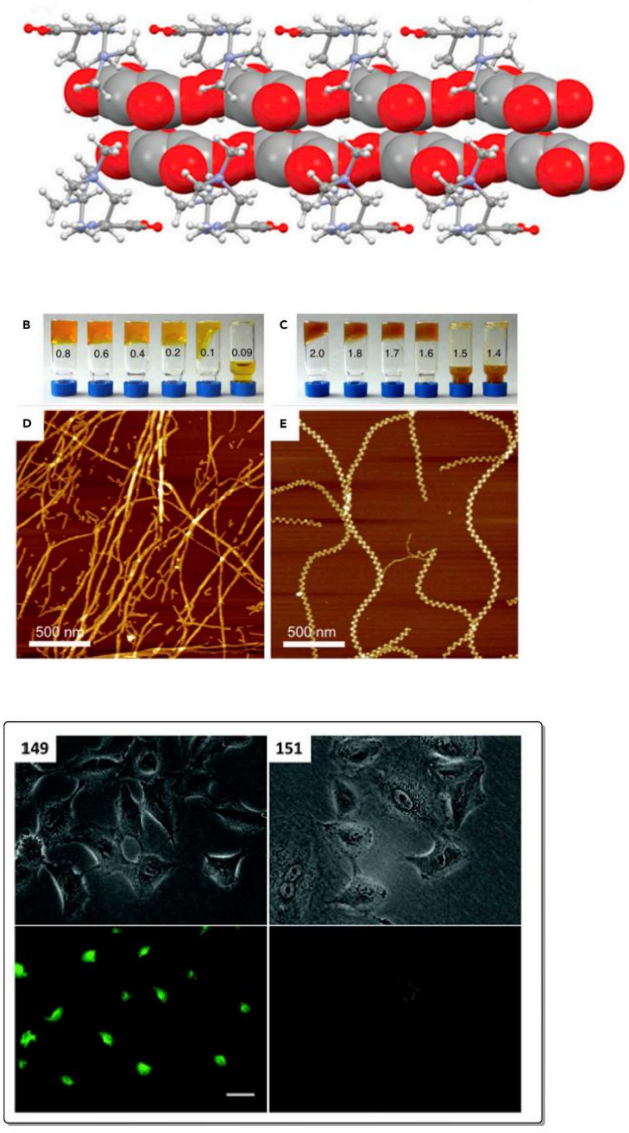

Figure 7. Fluorescent Squaramide-Based Anion Transporters

Squaric acid itself is known for the treatment of warts, and squaric acid dibutylester (SADBE) is also marketed as a treatment for alopecia. However, in more recent times, a number of squaramide-containing small-molecule drugs have entered clinical trials (Figure 49). Perzinfotel (EAA-090), a drug developed by Wyeth (now a wholly owned subsidiary of Pfizer), acts as a potent NMDA antagonist and was brought to phase 2 clinical trials for the treatment of neuropathic pain associated with diabetic neuropathy.116,117 Navarixin (MK-7123) is an antagonist of the cysteine-X-cysteine chemokine receptor 2 (CXCR2) and is under development by Merck. This compound was brought to phase 2 clinical trials for chronic obstructive pulmonary disease (COPD) and is currently being investigated in phase 2 clinical trials as a combination therapy against a range of metastatic solid tumors.118,119 Both Novartis and Boheringer Ingleheim have also reported squaramide-based drug candidates (156 and 157, respectively) for COPD treatment and even an early process-development study; however, despite multiple patent filings relating to CXCR2 antagonists, neither company appears to have progressed a candidate into

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development.120,121 Nevertheless, with such fervent squaramide-based research activity within industry, it is clear that the potential of squaramides is now being realized, and early-stage development of small-molecule drugs containing the squaramide motif has continued apace. Below, we review some of the more recent advances showing the versatility and potential of squaramide-based therapeutics across a broad range A team of scientists led by Ravishankar and Hameed at AstraZeneca recently reported the synthesis of potent squaramide-based inhibitors of mycobacterial adenosine triphosphate (ATP) synthase as a new approach to fighting tuberculosis infection.122 A structure-activity relationship (SAR) study for potency and selectivity identified a series of squaramide-based inhibitors, such as 158–160 (Figure 50), which were then evaluated in an ATP synthesis inhibition assay, where potencies in the nanomolar range were reported. Moreover, the lead compound, 160, was found to be extremely specific as an inhibitor of ATP synthase without any observed cytotoxicity and retained activity against a panel of drug-sensitive and single-drugresistant strains of Mycobacterium tuberculosis.

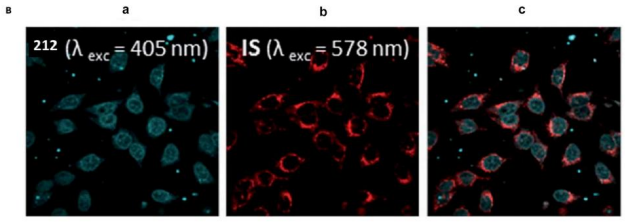

Figure 8. Luminescent Squaramide Monoesters for Cellular Imaging.

squaramides as an attractive scaffold for use across a diverse set of the chemical sciences. This review has attempted to give an overview of recent developments where major advances have been reported in the use of squaramides to construct large and complex self-assembly structures and materials, to catalyze a diverse set of synthetic transformations, to bind anionic guests, to sense anionic analytes, and most recently to stimulate transmembrane anion transport. Similarly, medicinal chemists have taken note of the scaffold, thought of as a bioisostere for biologically ubiquitous phosphate, where both academic research programs and pharmaceutical companies are pursuing new drug candidates containing the squaramide scaffold in a diverse range of therapeutic areas. The field of chemical biology is also gaining advantage from this useful class of compounds; for example, the sensitive and selective reaction of squarate esters with amines is being exploited as a

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mild and robust method for bioconjugation of peptides, proteins, carbohydrates, nucleosides, and fluorescent dyes. It is exciting to see such diverse development of squaramide chemistry, and this points to a natural evolution toward more applied uses of the scaffold. This class of simple organic building blocks has the potential to affect our lives through solving problems faced by the modern world; sensors for environmental pollution, new drug candidates, improved biomolecular assays, and novel functional materials are all possible. The future is bright for squaramides, and new and exciting developments are on the horizon as many more applications emerge and their true potential is fully realized.

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