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A concise review on application of squaric acid derivatives and their metal complexes in medicine

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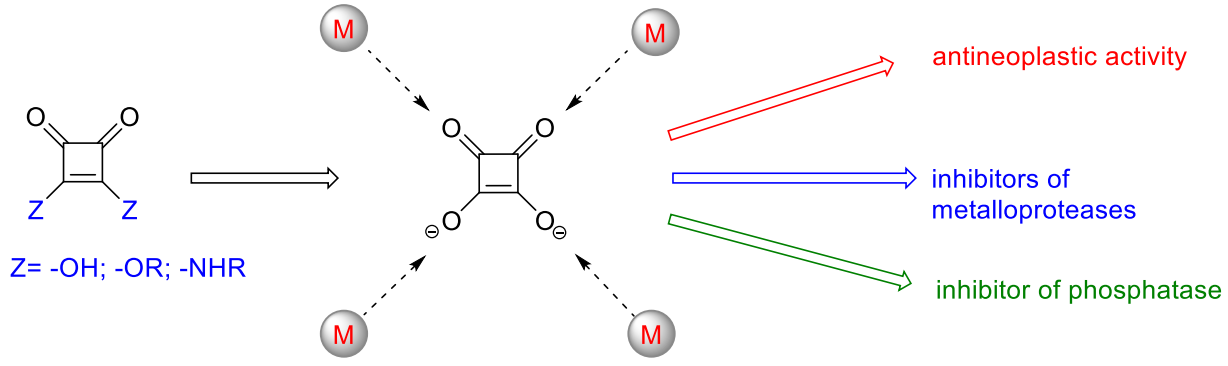
Abstract

In this review, we summarize the data about applications of squaric acid, its derivatives and their metal complexes in medicine. In recent years, researchers have been concentrated on the study of squaric acid and its derivatives because of their unique properties and the different possible ways to coordinate with metals and form complex compounds. Encouraging results have also been obtained for the cytotoxic activity of some squaric acid analogues and their metal complexes on various human tumor cell lines.



Keywords: *Squaric acid, squaramides, metal complexes, biological activity*

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1. Introduction

Over the years, researchers have been synthesizing and characterizing many metal complexes with squaric acid and its derivatives. All these complexes were studied for different applications like chemosensors, inhibitors, etc. But a few investigations on the application of squaric acid derivatives and their metal complexes were prepared and published. In 2020 Chasák et al.[1] published a review about the biological activity of squaric acid and its derivatives.

The focus of our review is on application of the squaric acid, squaramides and their metal complexes in medicine. Recently many researchers published synthesis and investigation of metal complexes of squaric acid and its derivatives. Furthermore, biological activity of the obtained metal complexes has been studied.

2. Squaric Acid and Oxocarbones - Characteristics

In 1960, West and co-workers identified a new class of organic compounds with cyclic structure and general formula $(C_nO_n)^{2-}$. These compounds were named oxocarbons.[2-5] The oxocarbon dianions (see Figure (1)) present a strong absorption in UV and visible regions with the exception of the deltate, which absorbs at wavelength lower than 200 nm. That raised the question “Are the oxocarbon dianions really aromatic?” The general conclusion is that the aromaticity decreases with the ring size. This conclusion is based not only on the symmetry shown from vibrational spectra, but also on the great stability of the anions, shown from the large dissociation constants of the corresponding acids. In crystal engineering, oxocarbon ions have been currently employed as building blocks, photoreceptors and semiconductor materials having non-linear optical properties (Figure 1).[6-11]

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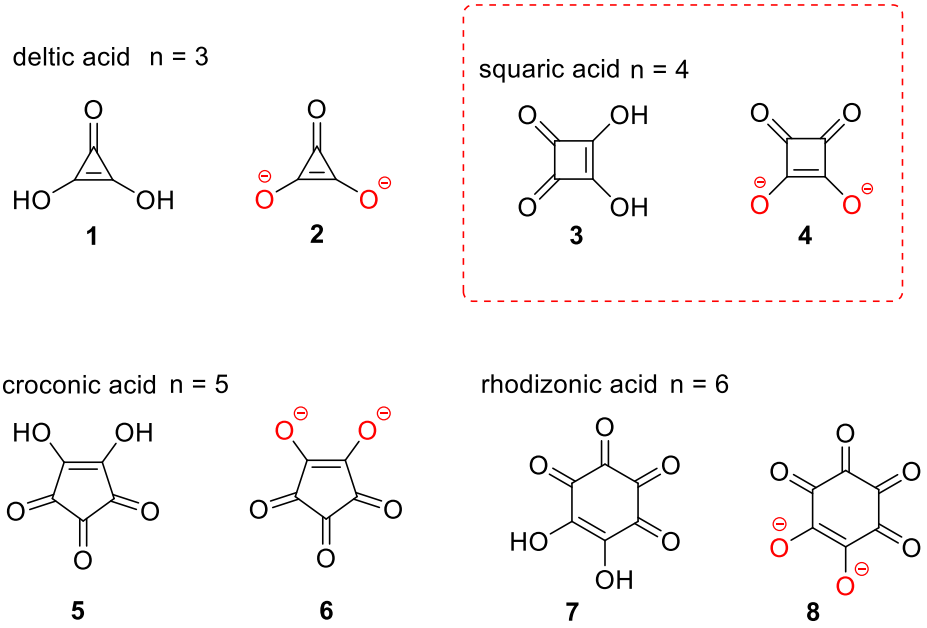

Figure 1. Chemical structures of oxocarbon acids and their dianions.

One representative of the group of oxocarbons is squaric acid (3,4-dihydroxycyclobut-3-ene-1,2-dione). In 1959 Cohen et al.[12] have obtained it for the first time and called quadratic acid because its four carbon atoms are forming square (Figure 2).

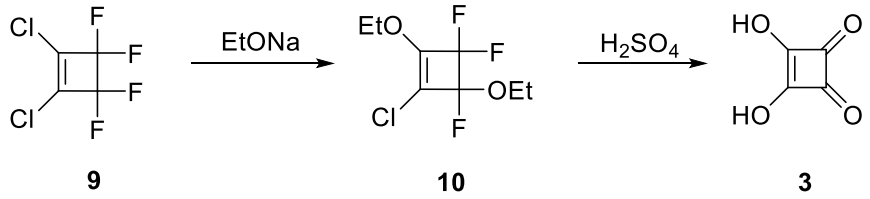

Figure 2. Synthesis of squaric acid by *Cohen et al.*

Later in 1974, Wang et a.l[13], showed by X-ray crystallography that the nonionized compound of squaric acid in reality is asymmetric, so in fact it forms trapezium. Samuelsen et al.[14], in 1975, examined the structure of the squaric acid and proved that it consists of planar layers of hydrogen bonded molecules. They determined that each molecule participates in four asymmetric hydrogen bonds, and thus there are two hydrogen atoms located nearest to anyone $(C_4O_4)^{2-}$ unit. The structure of the squaric acid allows the formation of intermolecular hydrogen bonds with carbonyl groups of proton acceptors and also formation of complex compounds with metal ions. This, in combination with its inherent high structural rigidity, allows squaric acid and its derivatives to have a variety of applications in organic chemistry, biomedical chemistry and material science. Squaric acid has a remarkable acidity, which is the highest among all cyclic oxocarbon acids and is explained by resonance stabilization of the dianion. It is a strong hydrogen-bonded and remarkably stable solid and forms clear crystals.[15-18] Squaric acid has a four-membered cyclic ring that exhibits two acidic hydroxyl groups, as well as two highly polarized carbonyl groups.[18-20]

Squaric acid can form mono- and dianions (Figure 3). It has two donor O–H groups along with two carbonyl acceptors, while its monoanion has one donor O–H group and three proton acceptors. All three

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species possess a certain degree of delocalization, but it is most pronounced in the dianion which is considered to be aromatic.[18-23]

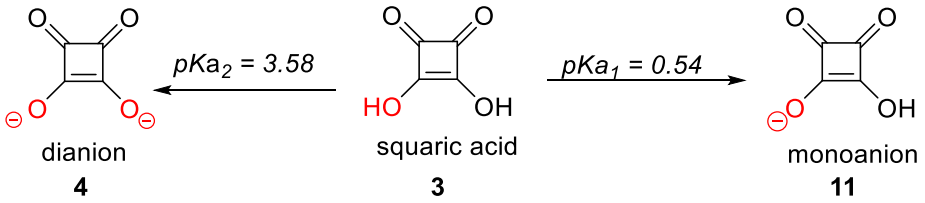

Figure 3. Squaric acid and its mono- and dianion.

Squaric acid versatility as a ligand is attributable to the fact that all four of its oxygen atoms are potential coordination sites, because they are chemically equivalent. This is due to resonance stabilization, leading to a fully delocalized aromatic structure. After losing two protons a squarate dianion $(C_4O_4)^{2-}$ is formed. It is a resonance-stabilized dianion owing to the delocalization of the negative charge over the C_4 ring and the four appended oxygen atoms. This delocalization is evidenced by the near equivalence of the respective C-C and C-O bond lengths of the squarate ion, except where hydrogen bonding causes a distortion resulting in a lowering of the symmetry from D_{4h}.[21-25]

The squarate dianion $[C_4O_4]^{2-}$ is water soluble and exhibits very unusual electronic and vibrational properties. It is a remarkable ligand in different coordination complexes, because all four oxygen atoms are potential coordination sites (Figure 4).

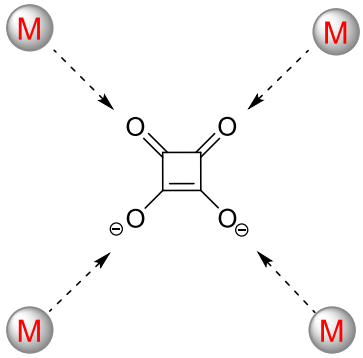

Figure 4. Coordination sites of squaric acid.

It has also been shown to participate in a range of different coordination modes. The squarate dianion can be both a chelate and a bridge ligand, such as a 1,2-bidentate chelate, 1,2-bis (monodentate) and 1,3-bis (monodentate) linking bridge. It has also been frequently used as a polyfunctional (μ1 to μ6 bridges) ligand that forms hydrogen bonding and π-π stacking interactions to form more extended networks.[26-33] X-ray diffraction studies by Oliveira et al.[33] have revealed all possible coordination modes of the squarate dianion with transition metals and lanthanides (Figure 5).

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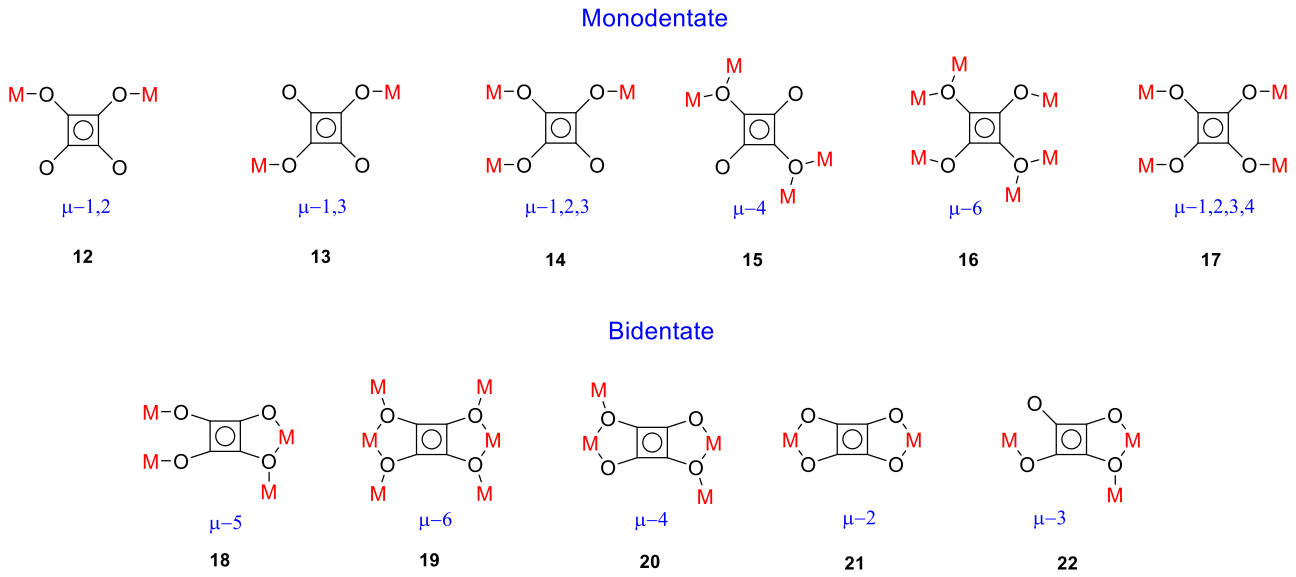

Figure 5. Schematic representations of all coordination modes of squarate dianion.

3. Squaramides and Their Application in Medicine

One group of amino derivatives of squaric acid is represented by squaramides. They have been known since 1950s, but have only recently emerged as particularly useful chemical entities in a variety of applications.

Squaramides are known to possess remarkable ring systems and structural rigidity[34] (Figure 6).

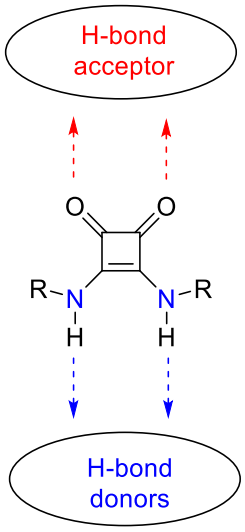

Figure 6. Representation of the possible hydrogen bonding sites in squaramides.

They are ditopic hydrogen-bonding synthons that can self-associate through two acceptors (C=O groups) and two donors (N–H groups) of hydrogen bond directly opposite one another on a cyclobutenedione ring.[34-35] Squaramides are used as coupling units for chelator and targeting vectors in radiopharmaceuticals for diagnostic and therapeutic purposes in nuclear medicine.[36] Chiral squaramides are powerful bifunctional hydrogen-bonding catalysts, and promoted numerous catalytic asymmetric transformations.[37]

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The squaramide-functional group has recently been exploited in supramolecular chemistry for the design of anion receptors. Elmes and co-workers[38] reported the synthesis of eight amino-acid based squaramide-anion receptors. They proved that the molecular properties of these receptors attenuated without affecting their anion recognition properties and this maybe will affect their anion recognition properties in biological or environmental applications. Busschaert et al.[39] reported for the anion transport properties of a series of squaramides and compared their transport abilities with analogous ureas and thioureas. This can be explained by the enhanced anion-binding properties of the squaramide-based receptors. In the last years very interesting results have been obtained for the realizing of asymmetric catalytic synthesis of nitro compounds in presence of small chiral organic molecules (organocatalysts).[40] Among the most efficient organocatalysts for enantioselective reactions of nitro alkanes and nitro alkenes are bifunctional chiral tertiary amines containing squaramide units.[41] Squaramides have also found wide applications in different annulation reactions.[42] Annulation reactions represent a powerful strategy for the construction of cyclic molecular frameworks. The asymmetric organocatalytic annulation reactions were used in total synthesis of natural products. Some novel squaramides have been prepared by Peng Li et al.[43] for the potential treatment of drug-resistant tuberculosis. The compounds displayed good to excellent *in vitro* antituberculosis activity and low cytotoxicity.

4. Application of Squaric Acid and Squaramides as Bioisosteres

Bioisosteric replacement is an important strategy to modify and optimize the properties of potential drugs. It may improve stability, optimize activity and minimize the side effects. [1,44-46] In 1950 Harris Friedman introduced the term "bioisosteres" and defined it as compounds eliciting a similar biological effect.[45]

Squaric acid and its derivatives can serve as a bioisostere of several functional groups, physiological important for medical chemistry and pharmacology. The sqaurate and sqauramide group can replace the pharmacologically important groups such as phosphate, carboxylate, sulphonate groups (Figure 7) because of the similar structural, electronic and physical properties like acidity, size of molecule, polarity, H-bonding.[48-54]

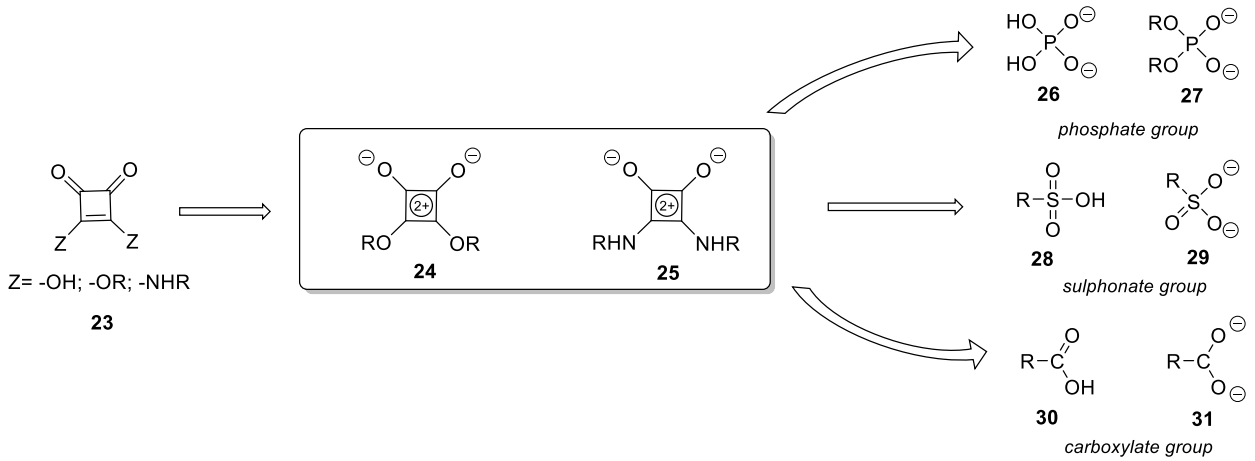

Figure 7. Squaric acid and its derivatives as bioisosteres.

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The electrostatic mimic of negatively charged groups squariamides as amino derivatives of squaric acid can used as bioisosters of urea, guanidine and thiourea (Figure 8) due to the similar formation of hydrogen bonds.[18,54,57-60]

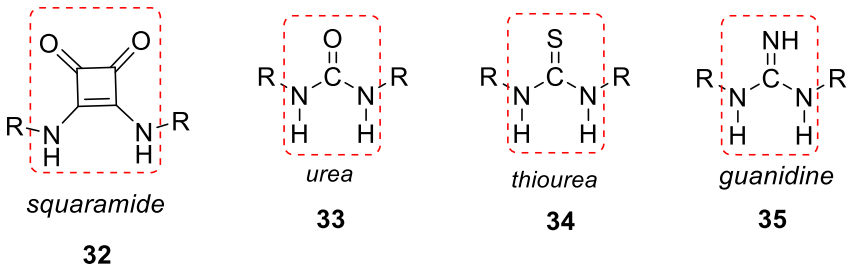

Figure 8. Squaramides as bioisosters.

Squaramides have rigid planar structures, so they exhibit 10–50 times greater affinity for halides than thiourea.[61] Urbahns et al.[62] have synthesized a variety of arginine bioisosteres, with one of them squaric acid amide, which mimicked urea fragments. The 3,4-diaminocyclobut-3-ene-1,2-dione fragment can replace the urea functionality since researchers at Wyetht, while working on the potassium channel openers (KCOs), have demonstrated that it is an effective replacement of a N-cyanoguanidine. Bioisosteric replacement of the N-cyanoguanidine moiety of the drug pinacidil with diaminocyclobutenedione template affords a novel series of bladder-selective potassium channel openers (KCOs).[56,63] Monosquaramide and disquaramide derivatives can serve as mimetics (isosteres) of amino acids based on structural homology, on the inherent strong dipole, and on electron density residing on the oxygen atoms. The 3,4-diamino-3-cyclobutene-1,2-dione group was described as a useful α-amino acid bioisostere.[46,56,64]

5. Application of Squaric Acid and its Derivatives in Medicine

It is known that various derivatives of squaric acid, substituted in a specific manner, have pharmacologically useful properties. The application of squaric acid and squaramide derivatives as anticancer agents has not been extensively studied, therefore no studies on various human tumor cell lines have been reported. [65-66] It has been shown that some squaramides selectively bind with protein kinases[66] or the CXCR2 receptor.[67] This shows that these compounds can selectively bind to cellular targets. Therefore it suggests that squaramides may serve as a good starting point for identifying the molecules which can specifically target cancer cells. Quintana et al.[68] studied a series of squaramides and squaramates for antitumor activity against different cancer cells that confirms the antitumor properties of this class of promising drug candidates. Two cancer cell lines were used – HeLa (cervical carcinoma) and HGC-27 (human gastric cell line). At first, preliminary screening was performed and then the most potent compounds were further evaluated. The data showed that HGC-27 cells seem to be more sensitive than HeLa cells to the effect of the tested squaramides.

Squaric acid and its derivatives have been studied for cytotoxic activity against a panel of human tumor cell lines especially against human leukemia cell lines. Liu *et al*. prepared series of novel 3,4-diaryl squaric acid analogues and studied their cytotoxic activity. Some of the new compounds exhibit strong cytotoxicity against human leukemia cells[57] (Figure 9). The cytotoxic activity may be due to the presence of three methoxy groups

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of A ring according to the SAR analysis results. For the design of structurally related tubulin inhibitors or combretastatin analogs the obtained results are very important.

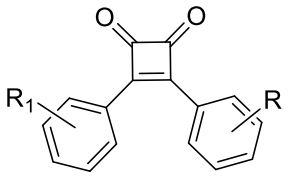

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Figure 9. General formula of cyclobutenedione derivatives.

Villalonga et al.[66] prepared some oligosquaramide-based macrocycles and discovered them as a novel class of kinase inhibitors with anticancer activity. The compounds tested showed noteworthy inhibition of kinases implied in cell proliferation such as ABL1 (ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase), CDK4 (Cyclin Dependent Kinase 4), PKC (Protein kinase C), c-Met (receptor tyrosine kinase), and FGF-R (fibroblast growth factor receptors). The data obtained expose that cyclosquaramides display cytotoxic activity on two mantle cell lymphoma (MCL) cell lines Jeko-1 and Z-138. The MCL is an aggressive subtype of non-Hodgkin lymphoma.

Some squaric acid derivatives were used for design of novel nucleoside analogues by Meijun Lu et al.[54] The squarate-based carboxylic nucleoside analogues 3-amino-4-((1R,4S)-4-(hydroxymethyl)cyclopent-2-en-1-yl)aminocyclobut-3-ene-1,2-dione, 3-((1R,4S)-4- (hydroxymethyl)cyclopent-2-en-1-yl)amino-4-methoxycyclobut-3-ene-1,2-dione, and 3-hydroxy-4-((1R,4S)-4-(hydroxymethyl)cyclopent-2-en-1-yl)amino-cyclobut-3-ene-1,2-dione sodium salt were synthesized. Computational analyses of their structures and preliminary antitumor and antiviral activities were reported. The compounds tested exhibited modest anticancer activities against non-small cell lung cancer (NCI-H522), ovarian cancer (OVCAR-8), leukemia (CCRF-CEM), renal cancer (UO-31), non-small cell lung cancer (NCI-H522), colon cancer (KM12), renal cancer (UO-31), melanoma (UACC-257), non-small cell lung cancer (NCI-H522) and of CNS cancer (SF-295) respectively.

4-Amino-3-[((1R,3S)-3-hydroxymethyl-4-cyclopentene)-1-amino]-3-cyclobutene1,2-dione, 3-methoxy-4-[((1R,3S)-3-hydroxymethyl-4-cyclopentene)-1-amino]-3-cyclobutene1,2-dione, 3-hydroxy-4-[((1R,3S)-3-hydroxymethyl-4-cyclopentene)-1-amino]-3-cyclobutene1,2-dionate sodium salts were synthesized and studied *in vitro* on 60 human tumor cell lines by Meijun Lu in the M.S. thesis. The new compounds were tested at a single dose of 10 μM and they did not exhibit significant growth inhibition. The same compounds were also tested for antiviral activity. The results showed that the compounds demonstrated reasonable antiviral and cytotoxicity profiles could be candidates for several additional follow-up analyses.[69]

A series of bioavailable 3,4-diaminocyclobutenediones with various amide modifications and substitution on the left side phenyl ring were prepared and found to show significant inhibitory activities towards CXCR2 (Chemokine (C-X-C Motif) Receptor 2) and CXCR1 (Chemokine (C-X-C Motif) Receptor 1) receptors.[70] The same group of researchers has also investigated a series of the 3,4-diaminocyclobutenediones with amide modifications and substitution on the right side phenyl ring. If the benzylic amine is keeping on the right side as constant, a number of amides were prepared and evaluated in the membrane binding CXCR2 and CXCR1 assays. The mono substituted alkyl amides showed weaker binding affinity towards the CXCR2 receptor compared to N,N-dimethyl amide that showed excellent inhibitory activity towards both receptors.[71]

The synthesis and testing of a series of substrate-mimic SNM1A (DNA cross-link repair 1A protein) inhibitors bearing squaramide or thiosquaramide ZBGs were reported by Berney et al.[72] Squaramides can

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chelate cations through their two carbonyl oxygen atoms.[73] Their derivatives as N-hydroxysquaramides have shown promise as ZBGs in inhibitors for metalloproteases.[74-75] It was shown that an oligonucleotide bearing a squaramide at the 5′-terminus is bound to SNM1A.[76] The use of squaramides as metal chelators in biological applications has not been fully explored yet. The compounds containing a squaramide group at the 5′-position proved to be ineffective, but some nucleoside derivatives with a squaramide moiety at the 3′-position demonstrated inhibition of SNM1A. The quantitative data showed that a thymidine derivative bearing a 5′-thiosquaramide was the most potent inhibitor, followed by a thymidine derivative bearing a 3′-squaric acid.

6. Application of Metal Complexes of Squaric Acid and its Derivatives as Cytotoxic Agents

Over the years, many articles about the synthesis and characterization of metal complexes with squaric acid and its derivatives were published but there is little data about their application in medicine. Squaric acid participates in the formation of large variety of complexes with transition metals because of the presence of four oxygen atoms.

The mode of coordination of squaric acid and its derivatives can be monodentate, bismonodentate, bidentate, trismonodentate and tetrakismonodentate. When the coordination is bismonodentate, it is often achieved by bridging either μ-1,2 or μ-1,3.[21]

Studies on the structures of alkaline earth squarates are very limited. K. T. Vadhana et al.[77] have synthesized and studied the following alkaline earth squarates, $\{[Ba(C_4O_4)(H_2O)_3]H_2O\}_n$, $\{[Sr(C_4O_4)(H_2O)_2]H_2O\}_n$, and $\{[Ba_{0.85}Sr_{0.15}(C_4O_4)(H_2O)_3]H_2O\}_n$. All complexes were tested *in vitro* for cytotoxicity against human breast cancer cell line MCF-7. The significant cytotoxic activity against the MCF-7 cell line is due to the carbonyl group, planarity and chelation of the organic ligand present in the metal complexes.[78-79]

Recently a great attention has been paid to the complexes containing 1,2-diaminocyclohexane (dach) as a ligand. Many researchers have used it and synthesized the complexes with squaric acid analogous as a second ligand. Zhang et al.[80] have prepared series of estradiol-derived Ni(II), Zn(II) and Pd(II) complexes containing a unique squaramide moiety (Figure 10).

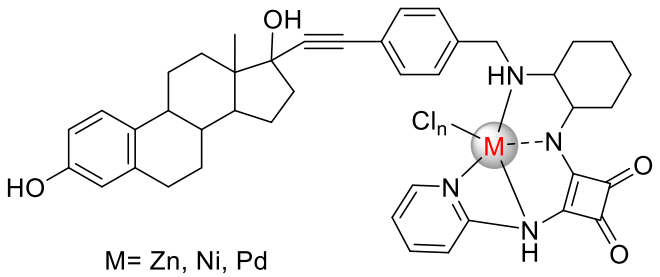

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Figure 10. Estrogen-derived steroidal metal complexes.

These authors have also examined the binding affinities of these compounds to the estrogen receptor-ligand binding domain (ER-LBD). The results showed effective binding of the compounds to the estrogen receptors. The compounds were also tested for transcriptional activity in human embryonic kidney 293T (HEK-293T) cells by a Luciferase reporter gene assays. All compounds synthesized were agonists on Erα in HEK-293T cells. In conclusion the tested compounds have showed low efficacy acting as antagonist on ERα.

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Since the squarate ion is chemically correlated to the oxalate ion, some platinum complexes with squaric acid instead of oxalate ion as a ligand were synthesized. Dioxycyclobutenedione-(1,2-cyclohexane-diamine)-platinum(II), (*cis*-[Pt(dach)(SA)], where SA is a dianion of squaric acid was reported by Yang and co-workers[81] (Figure 11). The complex has very close structure to that of oxaliplatin. It was studied for cytotoxic activity *in vitro* on six human tumor cell lines. The complex shows stronger cytotoxicity than cisplatin against human immature granulocyte leukemia (HL-60), erythroleukemia (K-562), human gastric carcinoma (BGC), human nasopharyngeal carcinoma (KB), human colon carcinoma (HCT) and human hepatocellular carcinoma (Bel-7402). The complex binds to DNA covalently, at the same way as that of cisplatin. The stronger cytotoxicity of *cis*-[Pt(dach)(SA)] than that of cisplatin is caused by its greater destruction to DNA than cisplatin.

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Figure 11. Chemical formulas of cis-[Pt(dach)(C_2O_4)] and *cis*-[Pt(dach)(SA)].

Zou et al. have synthesized and studied the uptake kinetics by human erythrocyte in the plasma isotonic buffer of Pt(II) complexes with different ligands and found that there is a correlation between the uptake rate and the carrier ligands of Pt(II) complexes. The uptake rate constant of [Pt(dach)X] is about 10 times higher than those of [Pt(NH_3)$_2$X]. The hydrophobicity of carrier ligands affects the intracellular accumulation.[82-83] Because the toxicity of the platinum complexes is dependent on the reactivity of the leaving groups, Zou *et all.* studied the relation between the toxicity and reactivity of the leaving groups in six new complexes with the general formulas [Pt(NH_3)$_2$X] and [Pt(dach)X] (where X is selenato anion, dianion of squaric acid (SA), or demethylcantharidin (DA)). The reactivity of leaving groups decreases in the sequence: *cis*-[Pt(NH_3)$_2$$Cl_2$] > *cis*-[Pt($NH_3$)$_2$(SA)] > *cis*-[Pt($NH_3$)$_2$(DA)] and for diaminocyclohexane platinum complexes (Figure 12).

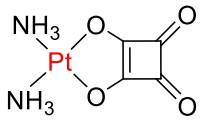

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Figure 12. Chemical formula of *cis*-[Pt(NH_3)$_2$(SA)].

Lialiaris and co-workers[84] synthesized and studied for biological activity binuclear platinum complexes with squaric acid that have biologically active squaric moiety and platinum active center in view to compare them with cisplatin. The goal was to prepare active anticancer agents by modification of the prototype cisplatin. In structures of the compounds there are ammonia molecule as a carrier ligand and chloride ion as a leaving group. The effect of the complexes on Sister Chromatid Exchange (SCE) rates and human lymphocyte proliferation kinetics was studied. SCEs have been proposed as a very sensitive method for detecting mutagens and/or carcinogens and lately as a method of evaluating chemotherapy *in vitro*[85] and *in vivo*[86].

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Studies have also shown that the determination of proliferation rates in lymphocyte cultures should be a useful and sensitive indicator of the cellular toxicity of chemotherapeutic agents.[87]. The complexes tested were $[Pt_2(NH_3)_2Cl_2(SA)]$ and $[Pt_2(NH_3)_4Cl_2(SA)]$. The results showed that the first compound is the most effective, on a molar basis in causing the cell division delays in comparison with the second one and cisplatin.

Squaramide motifs are class of interesting photoresponsive species. In 2018 Morales and co-workers[88] synthesized two squaramide-based Pt(II) complexes. They were evaluated for antiproliferative activity on HeLa cell line and compared with carboplatin. Complex $Pt(C_{12}H_{19}N_2O_2S_2)Cl$ is the first example of a platinum complex directly coordinated to a squaramide motif. It showed moderated cytotoxicity, whereas its irradiated form could not be evaluated because of its poor solubility. The $Pt(C_{12}H_{20}N_2O_2S_2)Cl_2$ complex is inactive on HeLa cells, but under hypoxic conditions, $C2'_H$ revealed remarkable enhancement of the antiproliferative activity that is in the same as of carboplatin (Figure 13).

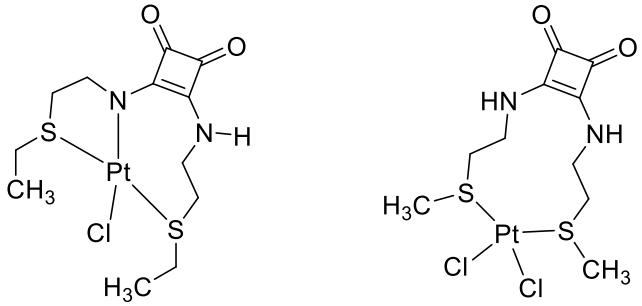

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Figure 13. Squaramide-based Pt(II) complexes.

Zinc hydroxamate squarate was presented by Onaran and co-workers[74] as a new inhibitor of metalloproteases (Figure 14).

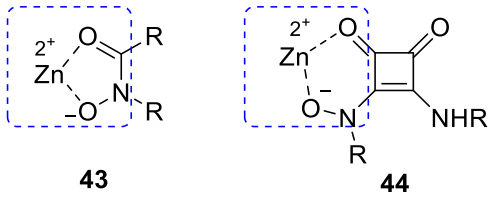

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Figure 14. Structures of zinc hydroxamate and zinc hydroxamate squarate.

Enzymes regulate structure and sustain a balanced composition of the extracellular matrix. Hydroxamic acids are commonly used as MMP inhibitors; Onaran et al presented for the first time squaric acid derivative as inhibitor of metalloproteases. Hydroxamic acid-based inhibitors are more potent than the squaric/hydroxamic acid hybrids. These hybrids could serve as an alternate starting point for the design of inhibitors with improved pharmacological properties.

Zhang et al. and Liu et al. developed a new vanadium complex as an inhibitor of phosphatase and tensin homologue (Figure 15).

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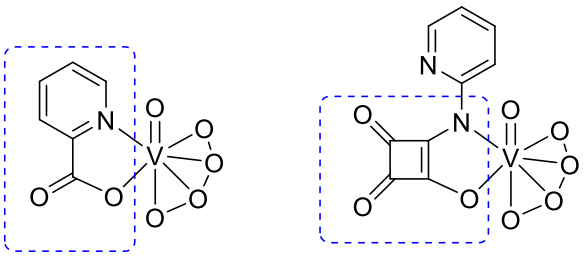

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Figure 15. Vanadium squaramide complex.

The squaramide complex has a neuroprotective effect and it could be gut mimic of the carboxylic acid and pyridine groups in this case.[89,90]

7. Conclusions

In this review, the literature data about the application of squaric acid, its derivatives and their metal complexes in medicine were described. A large number of squaric acid analogous were synthesized and studied for different applications. Squaric acid, squaramides, squaramates and other analogues have more than two coordination modes to bind with metals and formed complex compounds. Also, these compounds can be used as monodentate, bidentate, bridge ligands to form monuclear and binuclear metal complexes. However, little data about their application in medicine was published. In recent years the efforts of researchers were focused on studies of this class of organic compounds for cytotoxic activity, antiviral activity etc. The authors believe that this review will be very useful for researchers studying the application of the squaric acid analogues and their metal complexes in medicine.

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References

1. Chasák, J.; Šlachtová, V.; Urban, M.; Brulíková, L. *Eur. J. Med. Chem*. **2021**, 209, 112872–112923. https://doi:10.1016/j.ejmech.2020.112872
2. West, R.; Niu, H. Y.; Powell, D. L.; Evans, M. V. *J. Am. Chem. Soc*. **1960**, *82*, 6204.
3. Ito, M.; West, R. *J. Amer. Chem. Soc.* **1963**, *85*(17), 2580–2584. https://doi:10.1021/ja00900a011
4. Skujins, S.; Webb, G.A. *Spectrochim. A*cta A, **1969**, *25*, 917-924. https://doi:10.1016/0584-8539(69)80066-8
5. Farnell, L.; Radom, L.; Vincent, M. A. *J. Mol. Struct*. **1981**, *76*, l-10.

 ©**AUTHOR(S)**

https://doi:10.1016/0166-1280(81)85108-1
6. Aihara, J. *J. Amer. Chem. Soc*. **1981,** *103,* 1633–1635. https://doi.org/10.1021/ja00397a005
7. Schleyer, P. von R.; Najafian, K.; Kiran, B.; Jiao, H. *J. Org. Chem.* **2000**, *65,* 426–431. https://doi:10.1021/jo991267n
8. Junqueira, G. M. A.; Rocha, W. R.; De Almeida, W. B.; Dos Santos, H. F. *Phys. Chem. Chem.Phys*. **2001**, *3*, 3499–3505. https://doi:10.1039/b103975j
9. Junqueira, G. M. A.; Rocha, W. R.; De Almeida, W. B.; Dos Santos, H. F. *Phys. Chem. Chem. Phys.* **2002**, *4,* 2517–2523. https://doi:10.1039/b109738e
10. Seitz, G.; Imming, P. *Chem. Rev.* **1992**, *92,* 1227–1260. https://doi:10.1021/cr00014a004
11. Gicque, M.; Heully, J.-L.; Lepetit, C.; Chauvin, R. *Phys. Chem. Chem. Phys*. **2008**, *10,* 3578–3589. https://doi:10.1039/b718817j
12. Cohen, S.; Lacher, J.; Park, J. *J. Am. Chem. Soc.* **1959**, *81,* 3480. https://doi.org/10.1021/ja00397a005
13. Wang, Yu.; Stucky, G. D.; Williams, J. M. *J. Chem. Soc., Perkin Trans.* **1974,** *2,* 35–38. https://doi:10.1039/p29740000035
14. Samuelsen, E.J.; Semmingsen, D. *Solid State Commun.* **1975**, *17,* 217–219. https://doi:10.1016/0038-1098(75)90046-0
15. Zhou, L.; Zhang, Y.; Wu, L.; Li, J. *J. Mol. Struct. (Theochem)*, **2000**, *497,* 137–144 https://doi:10.1016/s0166-1280(99)00204-3
16. Schickinger, M.; Cibu, D.; Zischka, F.; Stierstorfer, K.; Jessen, C.; Kornath, A. *Eur. J. Chem.* **2018** https://doi:10.1002/chem.201802516
17. Wurm, F. R.; Klok, H.-A., *Chem. Soc. Rev*. **2013**, *42,* 8220–8237. https://doi:10.1039/C3CS60153F
18. Storer, R. I.; Aciro, C.; Jones, L. H. *Chem. Soc. Rev*. **2011**, *40,* 2330–2346. https://doi:10.1039/C0CS00200C
19. Sanz-Novo, M.; Alonso, E. R.; León, I.; Alonso, J. L. *Eur. J. Chem.* **2019,** *25*, 10748 – 10755. https://doi:10.1002/chem.201901916
20. Aknin, K.; Gauriot, M.; Totobenazara, J.; Deguine, N.; Deprez-Poulain, R.; Deprez. B.; Charton, J. *Tetrahedron Lett.* **2012**, *53(4),* 458–461. https://doi:10.1016/j.tetlet.2011.11.077
21. Hall, L.; Williams, D. J. *Adv. Inorg. Chem.*, **2001**, *52*, 249–291. https://doi:10.1016/s0898-8838(05)52005-1
22. Hopf, H.; Sherburn, M. S. *Cross Conjugation: Dendralene, Radialene and Fulvene Chemistry*, Hopf, H.; Sherburn, M. S. Eds.; Wiley-VCH Verlag GmbH & Co. KGaA **2016**, p.117–144. https://doi:10.1002/9783527671182.ch5
23. Allen, F. H.; Cruz-Cabeza, A. J.; Wood, P. A.; Bardwell, D.A. *Acta Crystallogr., Sect. B, Struct. Sci. Cryst. Eng. Mater.,* **2013**, *69,* 514–523. https://doi:10.1107/S2052519213020277
24. Tedesco, P. H.; Walton, H. F. Inorg. Chem. **1969**, *8(4),* 932–937. https://doi.org/10.1021/ic50074a044

 ©AUTHOR(S)

25. Li, S.-Ch.; Zhang, T.; Deng, X.-P.; Guo, X.-Q.; Zhou, L.-P.; Guo, F.; Sun, Q.-F. *Inorg. Chem. Commun*. **2018**, *92*, 68-73. https://doi:10.1016/j.inoche.2018.04.008
26. Solans, X.; Aguilo, M.; Gleizes, A.; Faus, J.; Julve, M.; Verdaguer, M. *Inorg. Chem*. **1990**, *29,* 775–784. https://doi:10.1021/ic00329a041
27. De Oliveira, L.F.C.; Santos, P.S. J. Mol. Struct. **1992**, *269,* 85–96. https://doi:10.1016/0022-2860(92)80007-5
28. Alleyne, B. D.; Hosein, H.-A.; Jaggernauth, H.; Hall, L. A.; White, A. J. P.; Williams, D. J. *Inorg. Chem***. 1999,** *38,* 2416–2421. https://doi:10.1021/ic981011k
29. Santos, P. S.; Sala, O.; Noda, L. K.; Gonçalves, N. S. *Spectrochim. Acta - A: Mol. Biomol. Spectrosc.*, **2000**, *56*, 1553–1562. https://doi:10.1016/s1386-1425(99)00278-4
30. Liu, C.-M.; Zhang, D.; Hao, X.; Zhu, D.-B. *ACS Omega*, **2016**, *1*, 286–292. https://doi:10.1021/acsomega.6b00083
31. Köferstein, R.; Robl, C. *Z. fur Anorg. Allg. Chem*. **2016**, *642,* 560–565. https://doi:10.1002/zaac.201600080
32. Lisnard, L.; Mialane, P.; Dolbecq, A.; Marrot, J.; Sécheresse, F. *Inorg. Chem. Commun*. **2003,** *6,* 503–505. https://doi:10.1016/s1387-7003(03)00028-5
33. De Oliveira, V. E.; Diniz, R.; Machado, F. C.; Oliveira, L. F. C., In Cross Conjugation: Dendralene, Radialene and Fulvene Chemistry, H. Henning; Sherburn, M. S. Edits. Wiley-VCH Verlag GmbH & Co. KGaA, 2016, pp 117- 144.
34. Han, X.; Zhou, H.-B.; Dong, C. *Chem. Rec.* **2016**, *16,* 897–906. https://doi:10.1002/tcr.201500266
35. Talens, V. S.; Davis, J.; Wu, C.-H.; Wen, Z.; Lauria, Fr.; Gupta, K. B. S. S.; Rudge, R.; Boraghi, M.; Hagemeijer, A.; Trinh, T. T.; Englebienne, P.; Voets, I. K.; Wu, J. I.; Kieltyka, R. E. *J. Am. Chem. Soc*. **2020**, *142,* 19907-19916. https://doi:10.1021/jacs.0c02081
36. Grus, T.; Lahnif, H.; Klasen, B.; Moon, E.-S.; Greifenstein, L.; Roesch F. *Bioconjugate Chem.* **2021**, *32*, 1223. https://doi.org/10.1021/acs.bioconjchem.1c00305
37. Li, P.; Hu, X; Dong, X.-Q.; Zhang, X. *Molecules* **2016**, *21,* 1327-1341. https://doi:10.3390/molecules21101327
38. Elmes, R. B. P.; Jolliffe, K. A. *Supramol. Chem.* **2015**, *27,* 321-328. https://doi:10.1080/10610278.2014.976221
39. Busschaert, N.; Kirby, I. L.; Young, S.; Coles, S. J.; Horton, P. N.; Light, M. E.; Gale, P. A. *Angew. Chem. Int. Ed.* **2012**, *51,* 4426–4430. https://doi:10.1002/anie.201200729
40. List, B.; Maruoka, K. Science of Synthesis: Asymmetric Organocatalysis, eds., Thieme: Stuttgart, 2012, vols. 1, 2.
41. Ananikov, V. P.; Khokhlova, E. A.; Egorov, M. P.; Sakharov, A. M.; Zlotin, S. G.; Kucherov, A. V.; Kustov, L. M.; Gening, M. L.; Nifantiev, N. E. *Mendeleev Commun*. **2015**, *25,* 75–82. https://doi:10.1016/j.mencom.2015.03.001
42. Wang, N.; Wu, Z.; Wang, J.; Ullah, N.; Lu, Y. *Chem. Soc. Reviews*, **2021**, *50,* 9766–9793.

 ©AUTHOR(S)

https://doi:10.1039/d0cs01124j
43. Li, P.; Wang, B.; Li, G.; Fu, L.; Zhang, D.; Lin, Z.; Huang, H.; Lu, Y. *Eur. J. Med. Chem*. **2020**, *206*, 112538-112552. https://doi:10.1016/j.ejmech.2020.112538
44. Meanwell, N. A. *J. Med. Chem.* **2011**, *54, 2529–2591.* https://doi:10.1021/jm1013693
45. Friedman, H.L. Influence of isosteric replacements upon biological activity, *First Symposium on Chemical Biological Correlation,* **1951**, *206*, 295-358.
46. Brown, N. *Bioisosteres in Medicinal Chemistry*, Brown, N. Edit., Wiley-VCH Verlag GmbH & Co. KGaA. **2012**, 1–14. https://doi:10.1002/9783527654307.ch1
47. Lassalas, P.; Gay, B.; Lasfargeas, C.; James, M. J.; Tran, V.; Viayendran, K. G.; Brunden, K. R.; Kozlowski, M. C.; Thomas, C. J.; Smith, A. B.; Huryna, D. M.; Ballatore, C. *J. Med. Chem.* **2016**, *59,* 3183–3203. https://doi:10.1021/acs.jmedchem.5b01963
48. Soukarieh, F.; Nowicki, M. W.; Bastide, A.; Poyry, T.; Jones, C.; Dudek, K.; Patwardhan, G.; Meullenet, F.; Oldham, N. J.; Walkinshaw, M. D.; Willis, A. E.; Fischer, P. M. *Eur. J. Med. Chem*, **2016**, *124*, 200–217. https://doi:10.1016/j.ejmech.2016.08.047
49. Marson, C. M. *Chem. Soc. Rev.* **2011**, *40,* 5514-5533. https://doi:10.1039/c1cs15119c
50. Shinada, T.; Hayashi, K.; Hayashi, T.; Yoshida, Y.; Horikawa, M.; Shimamoto, K.; Shigeri, Y.; Yumoto, N.; Ohfune, Y. *Org. Letters*, **1990**, *1,* 1663–1666. https://doi:10.1021/ol990286g
51. Lim, N. C.; Morton, M. D.; Jenkins, H. A.; Brückner, C. *J. Org. Chem*. **2003**, *68,* 9233–9241. https://doi:10.1021/jo035175g
52. Nottbohm, A. C.; Hergenrother, P. J. Phosphate Mimics: Cyclic Compounds. Wiley Encyclopedia of Chemical Biology, **2008**. https://doi:10.1002/9780470048672.wecb641
53. Onaran, M. B.; Comeau, A. B.; Seto, C. T. *J. Org. Chem.* **2005**, *70(26),* 10792–10802. https://doi:10.1021/jo0517848
54. Lu, M.; Lu, Q.-B.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **2017**, *2*, 282-287. https://doi:10.1016/j.bmcl.2016.11.058
55. Kitson, S. L. J. *Diagn. Imaging Ther.*, **2017**, *4,* 35-75 https://dx.doi.org/10.17229/jdit.20170503-029
56. Ohno, M.; Eguchi, S. *Bioact. Heterocycles,* **2006**, *I,* 1–37. https://doi:10.1007/7081_035
57. Liu, Z.; Wang, Y.; Han, Y.; Liu, L.; Jin, J.; Yi, H.; Li, Z.; Jiang, J-D.; Boykin, D.W. *Eur. J. Med. Chem.* **2013**, *65,* 187–194 https://doi:10.1016/j.ejmech.2013.04.046
58. Agnew-Francis, K. A.; Williams, C. M. *Chem. Revs*. **2020**, *120,* 11616–11650. https://doi:10.1021/acs.chemrev.0c00416
59. Seio, K.; Miyashita, T.; Sato, K.; Sekine M. *Eur. J. Org. Chem*. **2005**, *24*, 5163–5170. https://doi:10.1002/ejoc.200500520
60. Marchetti, L. A.; Kumawat, L. K.; Mao, N.; Stephens, J. C.; Elmes, R. B. P. *Chem*. **2019**, *5*, 1398–1485. https://doi:10.1016/j.chempr.2019.02.027

 ©AUTHOR(S)

61. Nagy, S.; Kisszékelyi, P.; Kupa, J. *Period. Polytech. Chem. Eng.* **2018**, *62*, 467-475. https://doi:10.3311/ppch.12851
62. Urbahns, K.; Härter, M.; Albers, M.; Schmidt, D.; Stelte-Ludwig, B.; Brüggemeier, U.; Vaupel, A.; Keldenich, J.; Lustig, K.; Tsujishita, H.; Gerdes, C. *Bioorg. Med. Chem. Letters*, **2007**, *17,* 6151–6154. https://doi:10.1016/j.bmcl.2007.09.039
63. Butera, J. A.; Antane, M. M.; Antane, S. A.; Argentieri, T. M.; Freeden, C.; Graceffa, R.; Hirth, B. H; Jenkins, D.; Lennox, J. R.; Matelan, E.; Norton, N. W; Quagliato, D.; Sheldon, J. H; Spinelli, W.; Warga, D.; Wojdan, A.; Woods, M. *J. Med. Chem.* **2000**, *43,* 1187–1202. https://doi:10.1021/jm9905099
64. Kinney, W. A.; Abou-Gharbia, M.; Garrison, D. T.; Schmid, J.; Kowal, D. M.; Bramlett, D. R.; Miller, T. L.; Tasse, R. P.; Zaleska, M. M.; Moyer, J. A. *J. Med. Chem.* **1998**, *41,* 236–246. https://doi:10.1021/jm970504g
65. Janetka, J. W.; Ashwell, S. *Expert Opin. Ther. Pat*. **2009**, *19*, 165–197. https://doi:10.1517/13543770802653622
66. Villalonga, P. ; Fernandez de Mattos, S. ; Ramis, G. ; ObradorHevia, A. ; Sampedro, A. ; Rotger, C. ; Costa, A. ChemMedChem, **2012**, *7*, 1472–1480. https://doi:10.1002/cmdc.201200157
67. Dwyer, M. P.; Yu, Y.; Chao, J.; Aki, C.; Chao, J.; Biju, P.; Girijavallabhan, V.; Rindgen, D.; Bond, R.; Mayer-Ezel, R.; Jakway, J.; Hipkin, R. W.; Fossetta, J.; Gonsiorek, W.; Bian, H.; Fan, X.; Terminelli, C.; Fine, J.; Lundell, D.; Merritt, J. R.; Rokosz, L. L.; Kaiser, B.; Li, G.; Wang, W.; Stauffer, T.; Ozgu, L.; Baldwin, J.; Taveras, A. G. *J. Med. Chem*. **2006**, *49*, 7603–7606. https://doi:10.1021/jm0609622
68. Quintana, M.; Allegre-Requena, J.V.; Marqués-López, E'.; Herrera, R.P.; Triola, G. MedChemComm **2016**, 7, 550-561. https://doi:10.1039/c5md00515a
69. Lu, M. Application of squaric acid to the preparation of bioactive compounds; a thesis presented to the University of Waterloo in fulfillment of the thesis requirement for the degree of Master of Science in Chemistry, **2008**.
70. Gonsiorek, W.; Fan, X.; Hesk, D.; Fossetta, J.; Qiu, H.; Jakway, J.; Billah, M.; Dwyer, M.; Chao, J.; Deno, G.; Taveras, A.; Lundell, D. J.; Hipkin, R. W. *J. Pharmcol. Exp. Ther*. **2007**, *322*, 477-
71. Aki, C.; Cha, J.; Ferreira, J. A.; Dwyer, M. P.; Yu, Y.; Chao, J.; Merritt, R. J.; Lai, G.; Wud, M.; Hipkinc, R. W.; Fan, X.; Gonsiorek, W.; Fosseta, J.; Rindgen, D.; Fine, J.; Lundell, D.; Taveras, A. G.; Biju, P. *Bioorg. Med. Chem*. *Lett.* **2009**, 4446–444919. https://doi:10.1016/j.bmcl.2009.05.049
72. Berney, M.; Doherty, W.; Jauslin, W. T.; Manoj, M. T.; Dürr, E-M.; Francelle, McGouran, *J. Bioorg. Med. Chem*. **202**1, *46*, 116369-11377. https://doi.org/10.1016/j.bmc.2021.116369
73. Quiñonero, D.; Frontera, A. ; Suñer, G. A.; Morey, J.; Costa, A.; Ballester, P.; Deyà, P. M. *Chem Phys Lett*. **2000**, *326*, 247–254. https://doi.org/10.1016/S0009-2614(00)00804-6
74. Onaran, M.B.; Comeau, A.B.; Set, C.T. *J Org Chem*. **2005**, *7*0, 10792–10802. https://doi.org/10.1021/jo0517848
75. Charton, J.; Déprez, B.P.; Déprez-Poulain, R.F. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 4968–4971. https://doi:10.1016/j.bmcl.2008.08.025

 ©**AUTHOR(S)**

76. Dürr, E.-M.; Doherty, W.; Lee, S. Y.; El-Sagheer, A. H.; Shivalingam, A.; McHugh, P. J.; Brown, T.; McGouran, J. F. *ChemistrySelect*. **2018**, *3*, 12824–12829. https://doi:10.1002/slct.201803375
77. Vadhana, K. T. P.; Parveen, S.; Ushadevi, B.; Selvakumar, R.; Sangeetha, S.; Vairam, S. *Acta Cryst*. **2019**, *C75*, 1091–1101. https://doi.org/10.1107/S2053229619009082
78. Kamatchi, S.; Chitrapriya, N.; Kim, S. K.; Fronczek, F. R.; Natarajan, K. *Eur. J. Med. Chem*. **2013**, *59*, 253-264. https://doi.org/10.1016/j.ejmech.2012.11.024
79. Sathiyaraj, S.; Sampath, K.; Butcer, R.; Pallapogu, R.; Jayabalakrishnan, C. *Eur. J. Med. Chem*. **2013**, *64*, 81-89. https://doi.org/10.1016/j.ejmech.2013.03.047
80. Zhang, X.; Zuo, Z.; Tang, J.; Wanga, K.; Wang, C.; Chen, W.; Li, Ch.; Xu, W.; Xiong, X.; Yuntai, K.; Huang, J.; Lan, X.; Zhou, H.-B. *Bioorg. Med. Chem. Letters* **2013**, *23*, 3793–37. https://doi.org/10.1016/j.bmcl.2013.04.088
81. Yang, M.; Pang, R.; Jia, X.; Li, Q.; Wang, K. *J. Inorg. Biochem*. **2005**, *99,* 376–382. https://doi:10.1016/j.jinorgbio.2004.09.028
82. Zou, J.; Dou, P. Y.; Wang, K. *J. Bioorg. Chem*. **1997**, *65,* 145–149. https://doi:10.1016/s0162-0134(96)00121-3
83. Zou, J.; Yang, X.; An,F.; Wang, K. *J. Inorg. Biochem.* **1998**, *70,* 227–232. https://doi:10.1016/s0162-0134(98)10020-x
84. Lialiaris, T.; Mourelatos, D.; Boutis, L.; Papageorgiou, A.; Christianopoulou, M.; Papageorgiou, V.; Dozi Vassiliades, J. *J. Pharmacol. Exp. Ther.* **1989**, *251,* 368-371.
85. Deen, D. F.; Kendall, L. E.; Marton, L. J.; Tofilon, P. J. *Cancer Res*. **1986, *46*,** 1599-1602**.**
86. Mourelatos, D.; Dozi-Vassiliades, J.; Kotsis, A.; Gourtsas, C. *Cancer Res*. **1988**, *48*, 1129-1131.
87. AbdeL-Fadil, M. R.; Palmer, C. G.; Heerema, H. *Mutat. Res*. **1982,** *104*, 267-273.
88. Morales, K.; Samper, K. G.; Peña, Q.; Hernando, J.; Lorenzo, J.; Rodríguez-Diéguez, A.; Capdevila, M.; Figueredo, M.; Palacios, Ò.; Bayón, P. *Inorg. Chem*. **2018***, 57,* 15517-15525. https://10.1021/acs.inorgchem.8b02854
89. Zhang, Z.-F.; Chen, J.; Han, X.; Zhang, Y.; Liao, H.-B.; Lei, R.- X.; Zhuang, Y.; Wang, Z.-F.; Li, Z.; Chen, J.-C.; Liao, W.-J.; Zhou, H.-B.; Liu, F.; Wan, Q. *Br. J. Pharmacol*. **2017**, *174*, 641−656. https://doi:10.1111/bph.13727
90. Liu, R.; Tang, J.-C.; Pan, M.-X.; Zhuang, Y.; Zhang, Y.; Liao, H.- B.; Zhao, D.; Lei, Y.; Lei, R.-X.; Wang, S.; Liu, A- Ch.; Qin, X.-P.; Chen, J.; Zhang, Z.-F.; Wan, Q. *Neurochem. Res*. **2018**, *43*, 1424−1438. https://doi:10.1007/s11064-018-2558-z

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