Original Article: Recent Biological Applications and Chemical Synthesis of Thiohydantoins

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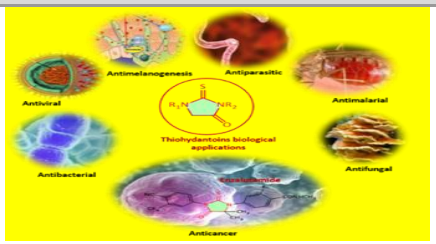
ABSTRACT

Thiohydantoins and their derivatives have attracted enormous attention from researchers worldwide. They exemplify compounds with bioactivity and therapeutic potentials. various pharmacologically active molecules within pharmaceutical chemistry are typical examples of thiohydantoins. Such compounds are studied to develop optimized drug treatments taking into account their chemical properties, the way they synthesized, and their common reactions. This review aims to place each work in the context of its contribution to understanding the significance of thiohydantoins and their derivatives



Introduction

Thiohydantoins are sulfur analogues of hydantoin (imidazolidine-2,4-diones) where one or both carbonyl groups were replaced by thiocarbonyl groups [1–5]. The backbone of thiohydantoin can be easily modified to adopt the preference structural type over another by adding groups to offer steric bulk, more hydrophilic or hydrophobic interactions, or π-π stacking. Therefore, their ability to form hydrogen-bonded arrays can be controlled in the solid-state, which is crucial in the pharmaceutical industry [6–9]. There are three thiohydantoins: 2-thiohydantoin (1) [10], 4-thiohydantoin (2) [11], and 2,4-thiohydantoin (3) (Figure 1) [12].

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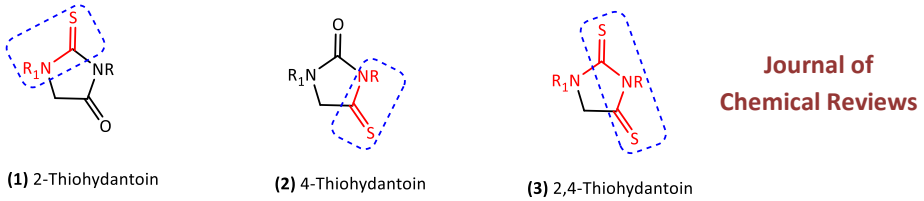


Figure 1. Chemical structure of thiohydantoins analogues

The well-known analogues are 2-thiohydantoins (2-thioxoximidazolin-4-ones) (1) due to their wide range of applications as intermediates and reagents in organic synthesis, pharmaceutical, and agricultural purposes. They are involved in the structure of many natural and synthetic molecules, playing important roles in the medical field. The nature of substituents on the heterocyclic ring affects the biological activity of these compounds. Position 5 in the five-membered ring 2-thiohydantoin is the nucleophilic centre, therefore, several biologically active 5-substituents 2-thiohydantoin derivatives can be prepared by condensation reaction at this position with various aldehydes (Figure 2) [13–16].

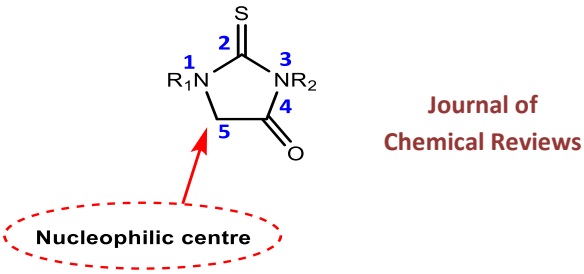


Figure 2. Chemical structure of 2-thiohydantoin core

Substitution of 2-thiohydantoin core at N-1 or N-3 or C-5 affects the properties of the molecule. Edward and Nielsen [17] studied the substitution impact of various alkyl and aryl groups on positions N-1, N-5, and C-5 in 2-thiohydantoins ring in three solutions (water, ethanol, and aqueous sodium hydroxide). Changes in ionisation and UV absorption occurred to 1-, 3- and 5-substituents alkyl or phenyl 2-thiohydantoins. Although these compounds are considered important scaffolds in drug discovery, few reviews have described their biological applications. Therefore, this review aims to discuss major biological applications of such compounds and the synthesis procedures and highlights their therapeutic activity.

Pharmacological Applications

The five-membered ring 2-thiohydantoins (1) exist in a wide spectrum of bioactive heterocyclic compounds. The biological activity of these compounds depends on the nature of substitution on the thiohydantoin ring. Among the pharmaceutical purposes that involve these active molecules the following:

Antitumor Activity

2-thiohydantoins and their analogues are among the anticancer agents that are presented as clinical candidates for treating different cancer types. A promising arylthiohydantoin derivative named enzalutamide (MDV3100, ENZ, 8) was chosen as a possible treatment for castration-resistant prostate cancer (CRPC), which has been used instead of bicalutamide (Bic) (4) due to its higher binding affinity to the androgen receptor (AR) than that for (Bic) as well as its ability to block nuclear transcription of AR [18]. Synthesis of this candidate based on the structure of both bicalutamide and the well-known nonsteroidal inhibitor (RU59063,5). The first generation included replacement of the hydroxy group at N1 by azidoalkyl group with different lengths of -CH2- linker (6) as well as another replacement by p-substituted aryl group (7) (Figure 3). After evaluation of the synthesised analogues, all of them demonstrated good activity against prostate-specific antigen (PSA) level higher than that of bicalutamide.

However, compound (7a) with azido p-aryl substituent exhibited the highest activity. The next generations based on modifications of generated molecules (7a-e). Several changes with various groups at positions N1, C4, and C5, which eventually led to the enzalutamide (8) [19]. Later in 2012, this molecule has been approved by FDA as a drug for CRPC treatment.

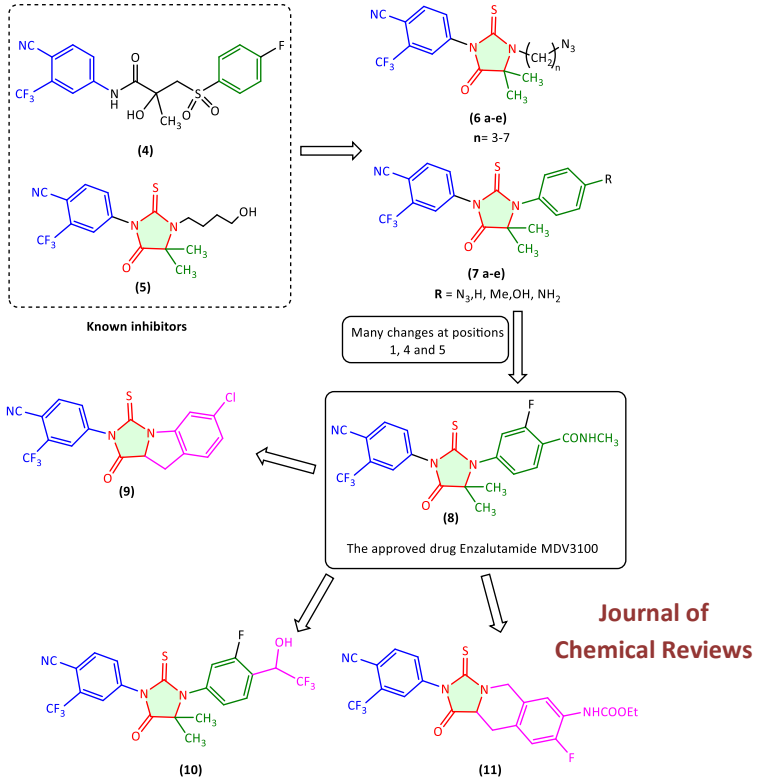


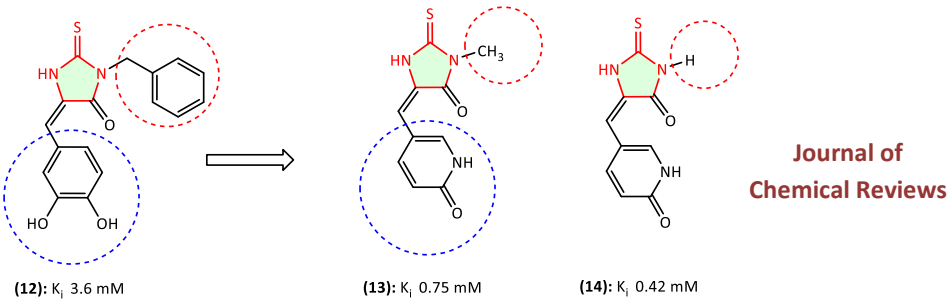
Figure 3. Chemical structure of androgen receptor antagonists.

Due to the developed genetic and phenotypic resistance to the enzalutamide by specific mutations of AR, such as F876L [20], different authors focused their research to discover novel antagonists by developing enzalutamide structure. In 2017, Zuo and coworkers reported indoline thiohydantoin (9) as a potent androgen receptor (AR) antagonist (Figure 3) [21]. The design of this novel scaffold based on the developments of the newly approved drug, enzalutamide (MDV3100, 8). After the SAR study of the enzalutamide, the authors suggested maintaining the 4-cyano-3-trifluorophenyl group attached to N2 in the thiohydantoin ring due to its critical role in the AR binding and they focused their attention on the modifications of the 3-fluoro-4-acetamidophenyl ring. A series of twenty-seven analogues were designed, synthesised and evaluated their activity against the androgen receptor. The most potent analogue was compound (9), which exhibited comparable antagonistic effect (IC_{50} 27.9 μM) with enzalutamide (IC50 12.5 μM) and less toxicity (> 200 μM) than that for enzalutamide (46.1 μM).

Wang et al. reported thiohydantoin analogue with 1-hydroxy-2,2,2-trifluoro-1-ethyl moiety at position N1 (10) as a novel inhibitor against androgen receptor through the design of a series of thiohydantoins by applying different modifications on the substituents at positions 3 and 4 on the phenyl ring at N1 as well as on position C5 [22]. They suggested replacing the amide group on the ring C of enzalutamide with its isostere 1-hydroxy and 1-amino-2,2,2-trifluoro-1-ethyl groups. After their synthesis, these compounds were evaluated for antiproliferative activity. According to the biological results, compound (10) was the most potent AR inhibitor, which is 2-fold more potent than ENZ with a low IC_{50} value (0.1 μM) against LNCaP-AR cell compared to that for ENZ (0.25 μM). In addition to its significant in vivo antitumor activity. In 2018, Xu and coworkers extended their previous work on indoline

thiohydantoin (9) by design a tetrahydroisoquinoline thiohydantoins scaffold [23]. The design was based on the incorporation of cycloalkane ring fused with a thiohydantoin ring. This has been introduced early in the same study reporting the high activity that was shown by these series. The compound with cyclohexane moiety (11) was a potent and selective androgen receptor antagonist, exhibited comparable inhibition against LNCaP-AR cell growth (IC_{50} 0.1 µM) and inhibition rate 85.1% with ENZ (IC_{50} 12.5 µM, inhibition rate 86.5%).

Recently, structure-activity relationship (SAR) investigation and biological evaluation of a novel series of synthesised 5-substituted 2-thiohydantion analogues led to a potent inhibition towards mutant isocitrate dehydrogenase (IDH1). This enzyme is involved in the tricarboxylic acid cycle by catalyzing the oxidative decarboxylation reaction of isocitrate to α-ketoglutarate and its mutations are associated with astrocytoma and oligodendroglial tumors (gliomas) [24]. 2-Thiohydantoin analogue (12) was considered a potent inhibitor of mutant IDH1 (R132H, Ki 4.7 µM). Efforts have been made to enhance the inhibitory activity of this compound by applying several modifications to its structure. These modifications included the replacement of groups on positions 3 and 5 in the thiohydantoin ring. Thirty replacements were performed on the parent compound (12). The SAR study and biological evaluation indicated that the most important group at position 5 was 2-pyridinon-5yl that exhibited a strong activity in the presence of a small group such as -H atom and -CH3 group at position 3 (Figure 4). Accordingly, the more potent inhibitors against IDH1 mutated cancers were compounds (14) and (13) respectively [25].



(12): K_i 3.6 mM **(13):** K_i 0.75 mM **(14):** K_i 0.42 mM

Figure 4. Chemical structure of the optimized IDH1 mutated cancer inhibitors

Antiparasitic activity

Buchynskyy *et al.* discovered the thiohydantoins 1-benzyl-3-aryl-2-thiohydantoins as anti-Trypanosoma brucei agents, species that cause Human African Trypanosomiasis (HAT, sleeping sickness). The analogue 1-benzyl-3-(3-chloro-4-methoxyphenyl)-2-thiohydantoin (15) was identified as a valid hit using high-throughput screening and hit validation. Structure-activity relationship (SAR) optimisation was performed on the hit compound to improve the inhibition efficiency of this molecule. Various changes at benzyl and aryl groups have been made leading to more potent inhibitors. Based on antitrypanosomal properties of the synthesised compounds towards parasites of Trypanosoma brucei species, two analogues 1-(4-fluorobenzyl)-3-(4-dimethylamino-3-chlorophenyl)-2-thiohydantoin (16) and 1-(2-chloro-4-fluorobenzyl)-3-(4-dimethylamino-3-methoxyphenyl)-2-thiohydantoin (17) (Figure 5) were displayed a high antiparasitic activity with IC_{50} 3.2 and 1.9 nM respectively [26,27]. Moreover, these potent inhibitors showed in vivo efficacy in an acute mouse model of human Trypanosomiasis by clearing parasite from mice blood.

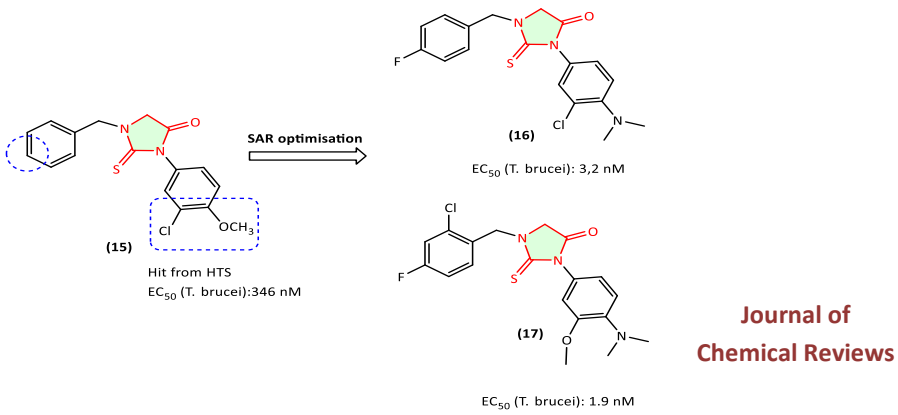
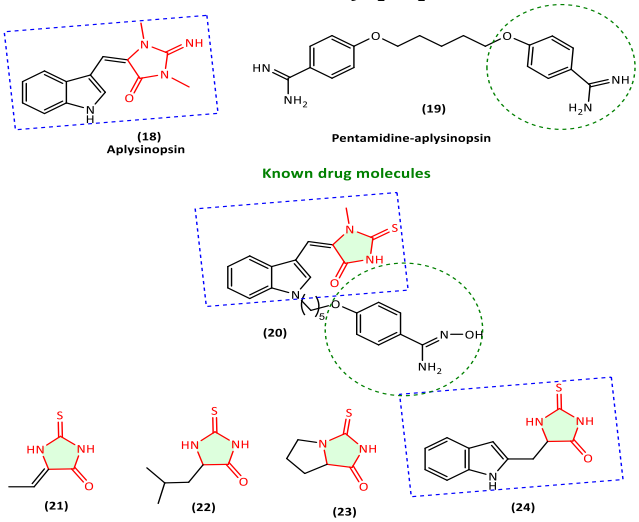


Figure 5. Chemical structures of HAT hits

In 2009, Porwal and coworkers published an article on the discovery of a novel thiohydantoin scaffold that contains E-configuration aplysinopsin as an antileishmanial agent (20) (Figure 6). This molecule Possesses a 2-thio analogue of aplysinopsin (18) as well as a p-guanidiniumphenoxy fragment from pentamidine (19), the well-known antileishmanial drug. The combination between these fragments in the lead structure improves both the activity and selectivity of the antileishmanial drug. The discovered compound displayed a promising inhibition toward parasite infection with IC_{50} = 2.0 μM, which is 10 times higher than that of pentamidine and its toxicity against human cells (108.5 μM) is less than that for the parent drug. However, the search for good and less toxic inhibitor is in demand [28]. Recently, Camargo et al. reported four thiohydantoins (21-24) derived from different L-amino acids namely glycine, valine, proline, and tryptophan based on the previous antileishmanial agent (20) (Figure 6). [29].



	IC50 (μM)a	Toxcicity IC50 (μM)b
(20)	2.0 ± 1.8	108.5 ± 4.1
(21)	9.97 ± 0.09	40.0 ± 0.13
(22)	10.40 ± 0.07	55.0 ± 0.07
(23)	9.31 ± 0.09	43.0 ± 0.10
(24)	10.46 ± 0.04	41.0 ± 0.18

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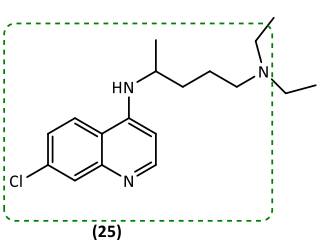
Figure 6. Discovery of new antileishmanial agents by modifications of known drugs. a Inhibition of promastigote forms of the L. amazonensis. b Concentration in μM cytotoxic for 50% of mammalian cells

Antimalarial

Malaria disease is caused by a pathogenic microorganism called Plasmodium falciparum and effectively treated with chloroquine scaffold drugs. Due to the rapid acquisition of these species to resist approved antimalarial drugs, the development of new medicine with a novel chemotype or mode of action for malaria treatment is required [30]. In 2014, Raghu Raj and coworkers reported the synthesis of a series of 7-chloroquinoline-thiohydantoins derivatives as antimalarial agents (Figure 7). These compounds were evaluated their inhibitory activity against P. falciparum and the potent compounds were also assessed their cytotoxicity against mammalian Hela cells. The most potent antimalarial activity were inhibitors (26 a-d) with promising low IC_{50} values of 39.84-57.21 nM and low toxicity. The effect of the length of the alkyl linker revers the potency, where the increase of alkyl chain length reduces the activity of the inhibitor [31].



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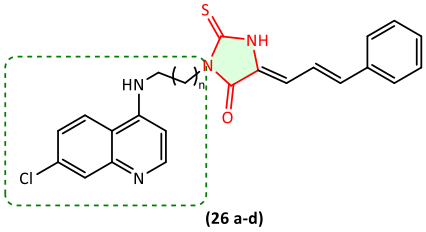
Marketed antimalaria drug
Cloroquine (CQ)



(26 a-d)

26a; n= 1, **26b;** n=2, **26c;** n= 3, **26d;** n=5

	p. falciparum IC_{50} (nM)	Cytotxcicity IC_{50} (μM)
26a	39.84	72 ± 6.22
26b	42.54	91 ± 8.12
26c	57.21	> 100
26d	386.9	
CQ	99.0	

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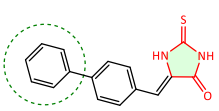
Figure 7. Structures of 7-chloroquinoline thiohydantoins hybrid possess chloroquine core.

Antibacterial

Compounds that have a thiohydantoin core in their structures possess antibacterial properties. In 2002, K. Kiec´-Kononowicz reported 5-arylidine-2-thiohydantoins (27 and 28) in Figure 30 as antimycobacterial agents. These compounds displayed more than 90% inhibition of Mycobacterium tuberculosis growth (97% for 27 and 95% for 28) with IC_{50} of 6.7 and 4.5 μM respectively. Their minimum inhibitory concentration (MIC) and selectivity index (SI) values were also measured. Compound 27 has a lower MIC (0.78 μg/ml) than that for compound 28 (1.56 μg/ml), while the SI values were (8.6 and 2.9) respectively [32].



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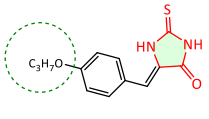

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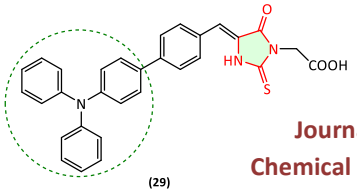

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Figure 8. Chemical structure of thiohydantoins with antibacterial activity

Recently, a series of 2-thiohydantoin derivatives containing triphenylamine moiety bounded through phenyl group linker in position C-5 and carboxymethyl substituent in N-3 position (Figure 8) has been designed based on previously reported antibacterial rhodanine-3-carboxylic acid with substituent triphenylamine at position C5 [33]. After these compounds were synthesised and evaluated their activity against selected bacterial strains from Gram-positive and Gram-negative as well as yeast, compound (29) was found to be the most antibacterial with low cytotoxicity against human cells (U-937, HUT-78 and COLO-720L) [34].

Antifungal activities

2-thiohydantoins possess fungicidal activity specifically those with 5-arylidine substituents. In 1993, Shdor Hosztafi and coworkers reported a series of 5-arylidene-2-thiohydantoins derivatives. Among these compounds, the molecule with dimethoxy phenyl at position 5 (30) (Figure 9) exhibited high fungicidal activity against Erysiphe graminis, Uromycceis appendiculatus, and Botrytis cinerea [35]. 5-Arylidene-2-thiohydantoin derivatives with a substituent sulfonyl group in position 4 as a linker between two phenyl groups (31 and 32) in Figure 9 were prepared and exhibited fungicidal activity higher than those in the case of the positive control (carbendazim) against Botrytis cinerea (inhibition rate 71.9%) and Alternaria solani (inhibition rate 57.6%) respectively [36].

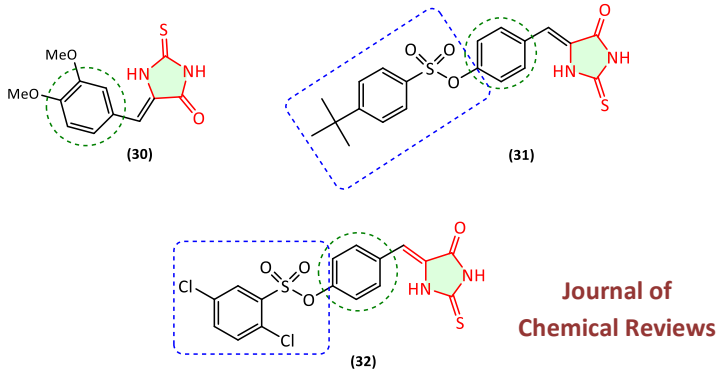

Figure 9. 2-thiohydantoins with Fungicidal activity

Antimelanogenesis

Melanin pigment is produced from L-tyrosine through a biosynthetic pathway catalyzed by different enzymes. The most important enzyme in this biosynthesis is tyrosinase, which is found in vertebrates, invertebrates, plants, insects, fungi, and bacteria [37,38]. Inhibition of this enzyme becomes an attractive subject including hyperpigmentation treatment, freckles, age spots, skin whitening agents, and melasma [39]. Moreover, tyrosinase inhibition plays an important role in the food industry, such as in the control of fruits and vegetable browning, which occurs due to the formation of quinone compounds by tyrosinase oxidative reactions. Therefore, by inhibiting tyrosinase, enzymatic browning reactions can be prevented. Many naturally occurring and synthetic compounds have been used as inhibitors of tyrosinase. The more recent inhibitors are arbutin and hydroquinone glycoside that is used in the cosmetic industry as whitening agents. However, these compounds are unstable over a long time, accordingly, the synthesis of more potent tyrosinase inhibitors that can be safely used in pharmaceutical, food, agriculture, and cosmetic industries are needed. In 2014, a series of 5-substituted benzylidene-2-thiohydantoin derivatives (36) (Figure 10) has been synthesised and evaluated against mushroom tyrosinase activity. These benzyledinethiohydantoin scaffolds were designed based on known potent inhibitors, benzyledinehydantoin (33), benzyledinepyrrolidinone (34), and benzyledinethiazolidine-2,4-dione (35). The most potent inhibitory activity was compound 2,4-dihydroxybenzylidene-2-thiohydantoin (36a) [40].

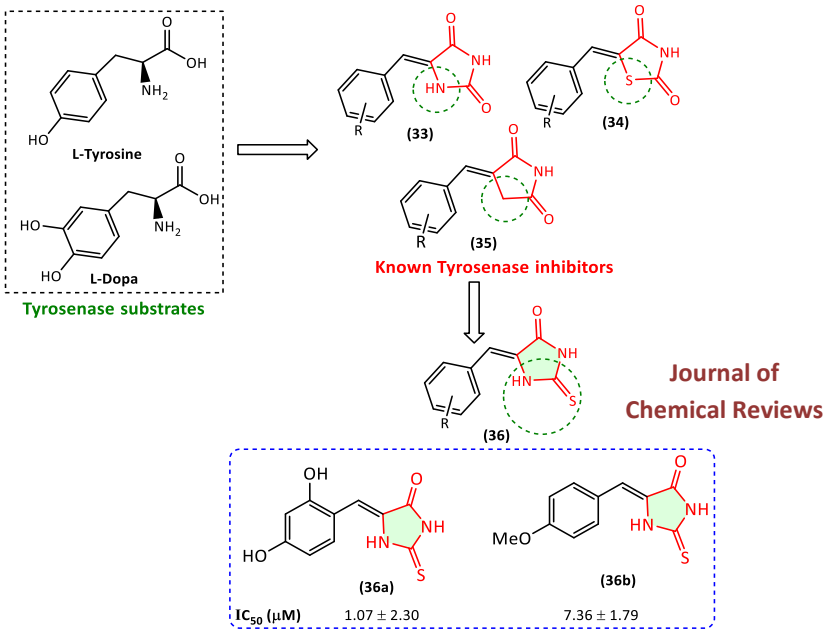

Figure 10. Chemical structure of synthesised 5-substituted benzylidene-2-thiohydantoin derivatives and their inhibitory activity towards tyrosinase

Other activities

In 2006, Michaux and coworkers reported 3-octyl-5,5-diphenyl-2-thiohydantoin (37) as a reversible and competitive inhibitor of fatty acid amid hydrolase (FAAH), a membrane-bound enzyme for signaling lipids hydrolysis, with IC_{50} of 6.1 ± 0.3 μM [41]. The mode of binding of this inhibitor to the rat FAAH active site was studied later by the same author [42]. To enhance the potency, the author and coworkers introduced a polar group at C-4 on the aromatic ring (38) (Figure 11) to bound the residues (Ser241, Ser217, and Lys142) in the FAAH catalytic active site. However, the efficiency of the new inhibitor (IC_{50} 23.4 ± 1.1 μM) was four times less than that for the original molecule [43].

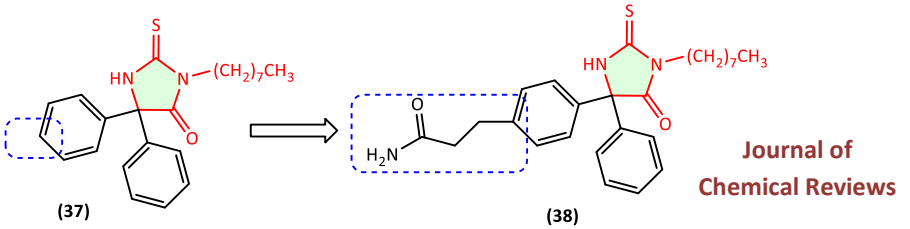

Figure 11. Representative structures of selected 5-benzyl 2-thiohydantoin derivatives as inhibitors of FAA

In 2016, Bae et al. reported the synthesis of a series of 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins as novel inhibitors for NOX (NADPH oxidases), enzymes that play critical roles in various pathologies, for example, vascular and fibrotic disorder, inflammation, and central nervous system diseases [44]. The design of these molecules based on the known NOX inhibitor, 3-phenyl-1-(pyridine-2-yl)-1H-pyrazol-5-ol (39) using molecular modelling. Taking into account the pharmacophore of the known inhibitor (39) and its binding mode with NOX, the suggested changes include hydroxypyrazole ring to thiohydantoin, and two aryl substituents to new aryl derivatives (Figure 33). The designed thiohydantoins were synthesised and then evaluated their inhibitory activity against NOX1 and NOX4. All synthesised compounds showed higher activity than that for the reference. In particular, thiohydantoin analogue (40) was the most potent inhibitor Ki values of 0.35 and 0.84 μM against NOX1 and NOX4.

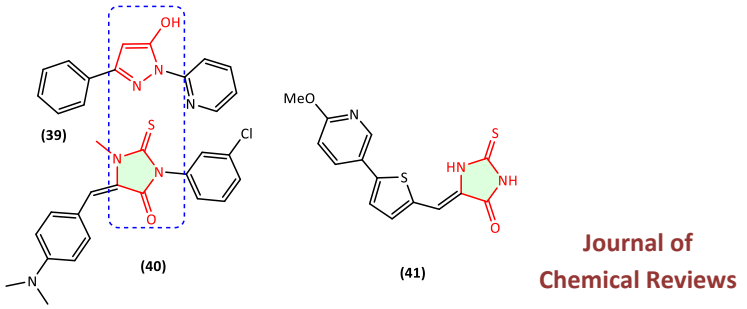

Figure 12. Design of benzylidene thiohydantoins inhibitors for NOX based on the known inhibitor

A library from 5-biarylidene-thiohydantoins was designed, synthesised, and tested their inhibitory activity against human and Leishmania Top1(DNA topoisomerase 1), the relax supercoiled DNA enzyme, by Majumdar and coworkers [45]. Among these compounds, 5(5-(2-methoxypyridin-5-yl) thiophen-2-ylidene) thiohydantoin (41, Figure 12) displayed a potent inhibition toward human Top1. Additional pharmacological evaluations were applied to this compound showed that it stabilises Top1 DNA cleavage complexes and selectivity exhibited anticancer activity against human breast (MCF-7) and cervical carcinomas (HeLa) cell lines.

Thiohydantoin as Catalysts in organic synthesis

Organocatalysts play a significant role in organic synthesis by catalyzing organic reactions that have limitations and improve the yield and stereoselectivity of some organic reaction products. Pyrrolidine is a five-membered ring secondary amine connected to other functional groups producing effective organocatalysts that catalyze various asymmetric transformations, such as sulfonamide [46], chiral diamines [47], diarylprolinols [48]. Recently, the pyrrolidine ring has been combined with the thiohydantoin ring to improve the organocatalyst “priviledge" that catalyzes asymmetric Michael addition. Five analogues of “priviledge" were prepared from five 2-thiohydantoin derivatives with different substituents in position 5 and pyrrolidine. The catalytic activity of these catalysts was tested for the reaction of cyclohexanone (43) with trans-β-nitrostyrene (44). The catalyst with the t-But group in position 5 in the thiohydantoin ring (42b) led to the highest quantitative yield and high stereoselective products (45) (Figure 13) [49].

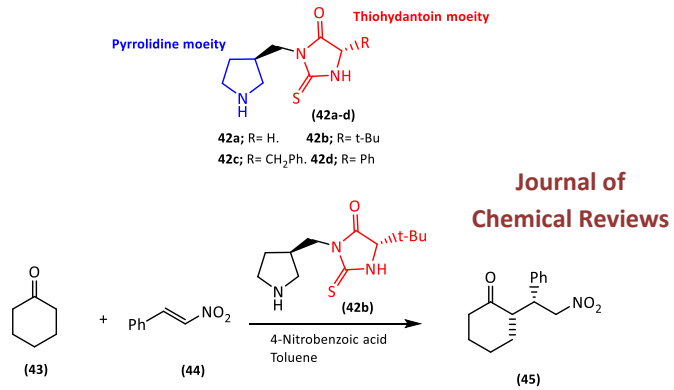

Figure 13. Michael’s reaction by using 2-thiohydantoin derivative as a catalyst

Synthesis of 2-thiohydantoins

Due to the biological importance of 2-thiohydantoins, different methods have been used to prepare various derivatives from these compounds. The most common approaches are based on the reaction of α-amino acids with thiocyanate or isothiocyanate derivatives. Johnson et al. reported the synthesis of 2-thiohydantoin from the reaction of acyl α -amino acid derivatives such as aceturic acid (46) and hippuric acid (46) with thiocyanate in acetic

anhydride (Figure 14). The suggested reaction mechanism was based on the formation of azlactone (47) as an intermediate by a nucleophilic attack of the carboxyl group at activated carbonyl acetyl through intramolecular reaction. Thiocyanate attacked the carbonyl ester in the azlactone ring causing ring-opening, which then undergoes intramolecular reaction afforded thiohydantoin ring (49) [1–3,10]. Ammonium thiocyanate is not the preferable reagent in this reaction as reported by Inglis et al. since a strong acid is required [50].

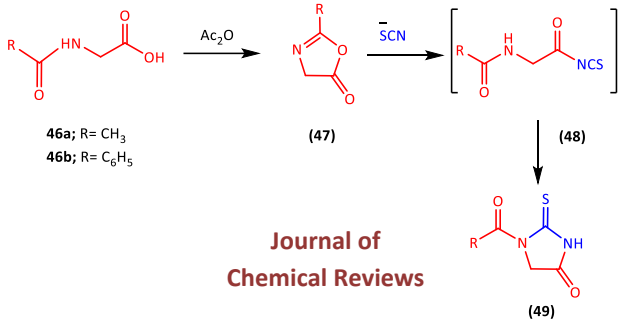

Figure 14. A suggested mechanism for the formation of 2-thiohydantoins.

Thiohydantoins prepared by this procedure did not mention their optical activity by authors. However, the synthesis of D-1-acetyl-5-methyl-2-thiohydantoin from D-alanine has obeyed a similar way that reported by Csonka and Nicolet [51].

Again, the same procedure has been applied for various N-substituted amino acids (50 a-e), which were formed from the reaction of α-halo α-substituted carboxylic acids with different amines or by reductive amination of glyoxylic acid (53). Additionally, in the case of 50a, a treatment with amines is needed. The cyclisation step of the intermediate (51) was performed under basic conditions from triethylamine afforded thiohydantoins (52) (Figure 15) [52].

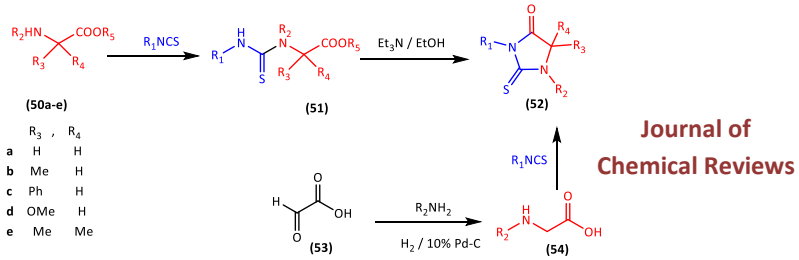

Figure 15. Synthesis of 2-thiohydantoins from N-substituted amino acids.

Sim and Ganesan [53] reported an efficient one-pot three components procedure, which included a reaction between α-amino acid ester (55) and aldehyde (56) followed by reduction of resulted imine (57). Isothiocyanate (58) was then added to the reaction mixture afforded 2-thiohydantoin derivatives (59) (Figure 16).

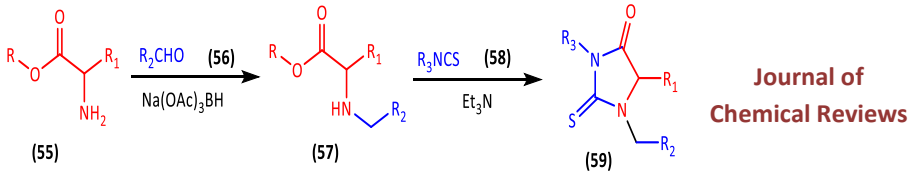

Figure 16. Combinatorial synthesis of 2-thiohydantoin derivatives.

Instead of using amino acids and their derivatives, cyanoamine (62) has been reacted with isothiocyanate to generate thiohydantoin-4-imine (63), which then hydrolysed by mild acidic conditions to the 2-thiohydantoin derivative (64) (Figure 17). The preparation of cyanoamine was by reaction of ketone (61) with an amine in the presence of trimethylsilyl

cyanide [54,18] or by treatment of cyanohydrin (60) with amine as previously reported [55].

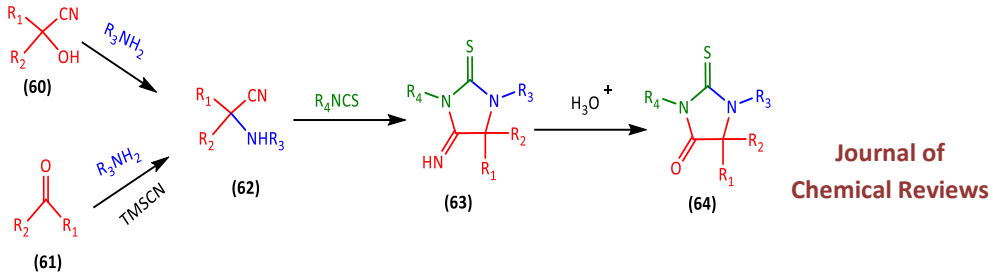

Figure 17. Synthetic procedure for 2-thiohydantoins from amino cyanide.

Thiourea and its derivatives were also used to synthesis the bioactive 2-thiohydantoins analogues under microwave-assisted conditions. In 2003, Muccioli and coworkers synthesised a new 2-thiohydantoin derivative named N3-ethyl-5,5'-diphenyl-2-thiohydantoin (67) by the reaction of thiourea derivative (66) and benzile (65) under microwave conditions and through rearrangement reaction of the formed intermediate (Figure 18) [56].

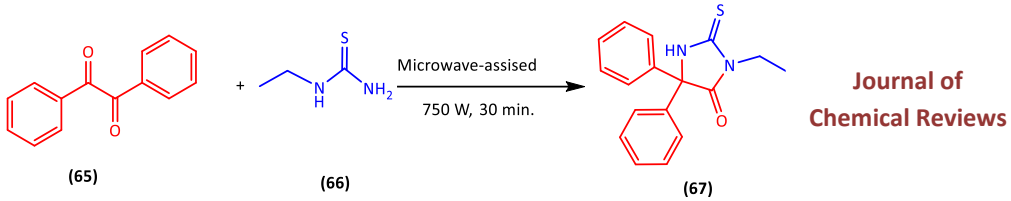

Figure 18. Microwave-assisted synthetic route for diphenylthiohydantoin UIU9GFY P]; DX synthesis.

In 2006, Wang and coworkers reported the condensation reaction of thiourea (69) with α-amino acids (68) to produce 2-thiohydantoins (70) under heating conditions (Figure 19) [57]. This procedure was successfully applied on the most natural α-amino acids including L-proline which previously failed at conversion into 2-thiohydantoin derivative.

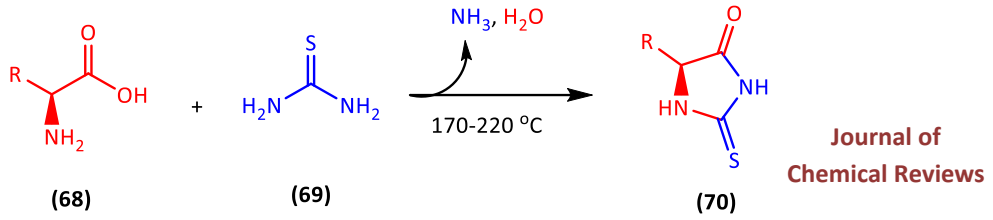

Figure 19. The general procedure for 2-thiohydantoins synthesis from thiourea and α-amino acids.

Bucherer-bergs reaction, which involved four components (4RC) has been modified by Montagne et al. [58]. The modification was to synthesize hydantoin derivatives using nitrile compounds as starting material instead of ketones (Figure 20). This procedure was utilized for 2-thiohydantoins synthesis starting with organometallic reagent n-BuLi in the first step treated with 1,4-diiodobenzene (71), followed by the addition of nitrile compound (PhCN) and potassium cyanide producing 5,5'-disubstituted hydantoin (72). The alkylation reaction at N-3 of this product has been selectively achieved in the next step afforded followed by a coupling reaction between this product and methyl acrylate under microwave conditions. For the transformation of hydantoin to thiohydantoin, Lawesson's reagent was successfully used to produce thiohydantoin intermediate (73). Biologically active compounds were successfully formed by the intermediate (74) [59,43].

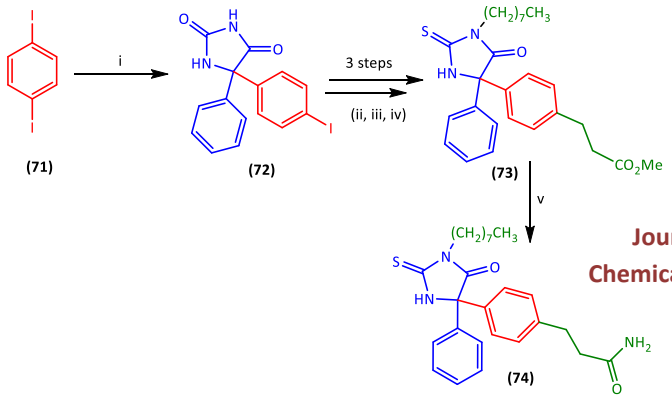

Figure 20. Synthesis of 2-thiohydantoins derivatives from phenyl iodide. Reagents: i) (1)- n-BuLi, THF, -78 ºC. (2)- PhCN, -78 -0 ºC. (3)- KCN, $(NH_4)_2CO_3$, 70 ºC, EtOH / H_2O (1:1), µW. ii) K_2CO_3, DMF, $Me(CH_2)_7Br$. iii) $MeO_2CCH=CH_2$, $Pd(OAc)_2$, Bu_3N, DMF, 100 ºC, µW. iv) (1)- H_2, Pd/C, MeOH, r.t. (2)- Lawesson's reagent, PhMe, 110 ºC. v) (1)- LiOH, THF/H_2O (1:1). (2)- NH_3, HOBt, PyBO, DMF, r.t.

In 2018, Soural et al. reported a convenient procedure using solid phase synthesis to produce novel 2-thiohydantoins (Figure 21). The reaction was started by forming α-acylamino ketones (76) from wang resin bound, Fmoc-Ser(t-Bu)-OH (75) through four steps. Four sequence reactions were applied on this starting material include, thioamide formation (77), Fmoc deprotection (78), spontaneous cyclative cleavage, afforded 2-thiohydantoins analogues (79), the final reaction was hydrolysis of the product with trifluoroacetic acid (TFA) to produce the oxazine (80). However, the hydrolysis result was the product (81), which then treated with TFA in the presence of triethylsilane (TFA/TES) to reduce the methylene group and gave the final product 5-methyl-2-thiohydantoins (82) [60].

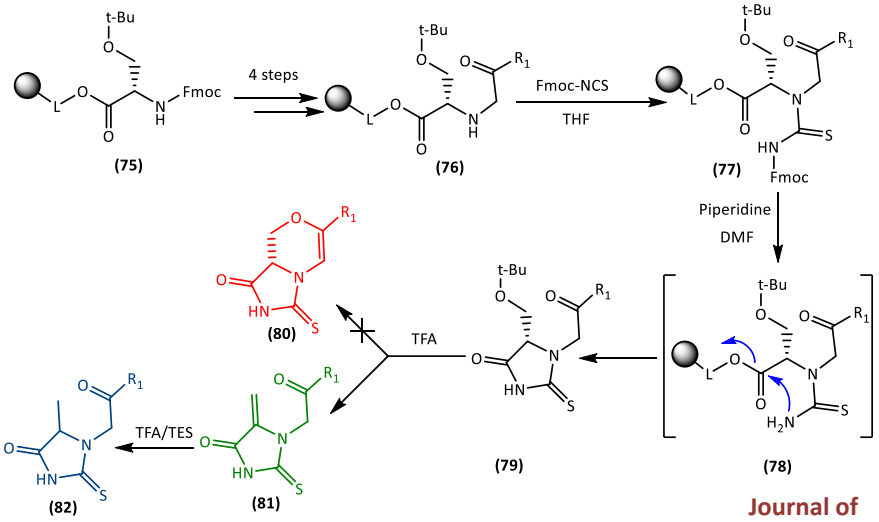

Figure 21. Solid phase synthesis of 2-thiohydantoin derivatives

Reactions of 2-thiohydantoins

Hydrolysis catalyzed reaction of 2-thiohydantoins

Edward and Nielsen evaluated the hydrolysis of 5-substituted 2-thiohydantoin in alkaline solution [61]. In the first step of this base-catalyzed hydrolysis, 2-thiohydantoin (83) was ionized rapidly to the ion (84). Meanwhile, a slow hydrolysis reaction occurred afforded thioureido-acids (87) through the intermediates (85) and (86) according to the suggested mechanism (Figure 22). The supportive evidence of this hydrolysis is the low productivity of 2-thiohydantoins in the established Schlack and Kumpf's protein sequencing method [62].

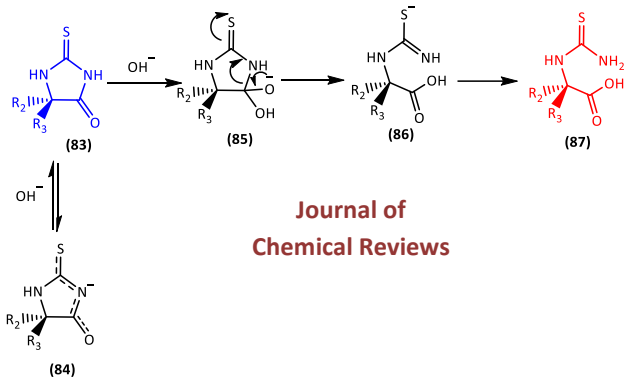

Figure 22. Mechanism of base-catalysed hydrolysis proposed by Edward and Nielsen

Substituted 1-acetyl-2-thiohydantoin ring can be cleaved by acid or base catalysts. The acyl group at position N-1 in 1-acyl-2-thiohydantoin (88) degrades under mild acid or alkaline conditions to 2-thiohydantoin (90) similar to the case of amides [63,64] and esters [65, 66]. This degradation is related to peptides or proteins degradation previously reported by Schlack and Kumpf [67] where the C-terminal of polypeptide or protein reacted with ammonium thiocyanate produced a 2-thiohydantoin derivative attached to the peptide chain by 1-acetyl linkage as shown in Figure 23.

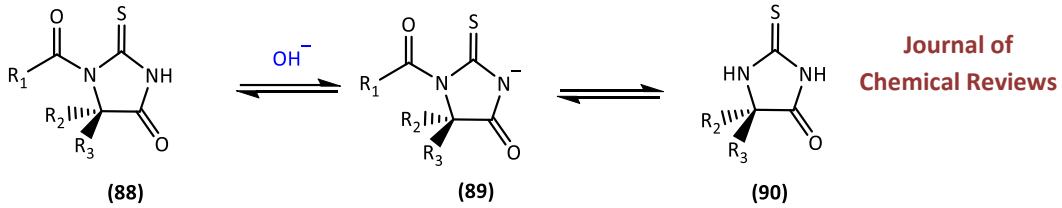

Figure 23. Cleavage of acetyl linkage attached to the thiohydantoin derivative in position N-1 by base-catalysed hydrolysis

Congdon and Edward reported the reaction mechanism of base-catalyzed hydrolysis of 5-substituted 1-acyl-2-thiohydantoins (88) (Figure 23) [68]. After ionization of N3 under basic conditions as a weak acid and has pKa values of 6.5-7.0. However, the negative charge in the generated anion does not delocalized over the acetyl group, therefore, the reactivity is expected to decrease. Following, the acyl group left as a carboxylic acid after the nucleophilic attack of hydroxide ion [68] on the anion (1) produced 2-thiohydantoin (9) via three intermediates (92, 93, and 94). In the case of acid hydrolysis, the reaction started by protonation of the acyl group (95), followed by nucleophilic attack of a water molecule on the acyl group (6). In the next step, the acyl group cleaved also as a carboxylic acid afforded 2-thiohydantoin (9) through the intermediate (7).

The substituents at position C-5 affect the acid and base-catalyzed hydrolysis by slow down the basic hydrolysis much more than acid-catalyzed hydrolysis. The possible suggested reason is the steric hindrance in the alkaline hydrolysis by the substituent groups at position C-5, which prevents the formation of tetrahedral intermediate (2) due to the hydrogen bonding between the new hydroxyl group and the strong basic sulfur atom. However, in acid-catalyzed hydrolysis, this effect is small due to the difference in the charge center in the intermediate (5). The rate of base-catalyzed hydrolysis influences by the ionic strength and the pH of the buffer. Therefore, in strong alkaline solutions, the hydrolysis to 2-thiohydantoin and carboxylic acid occurs rapidly [61,66,68,69].

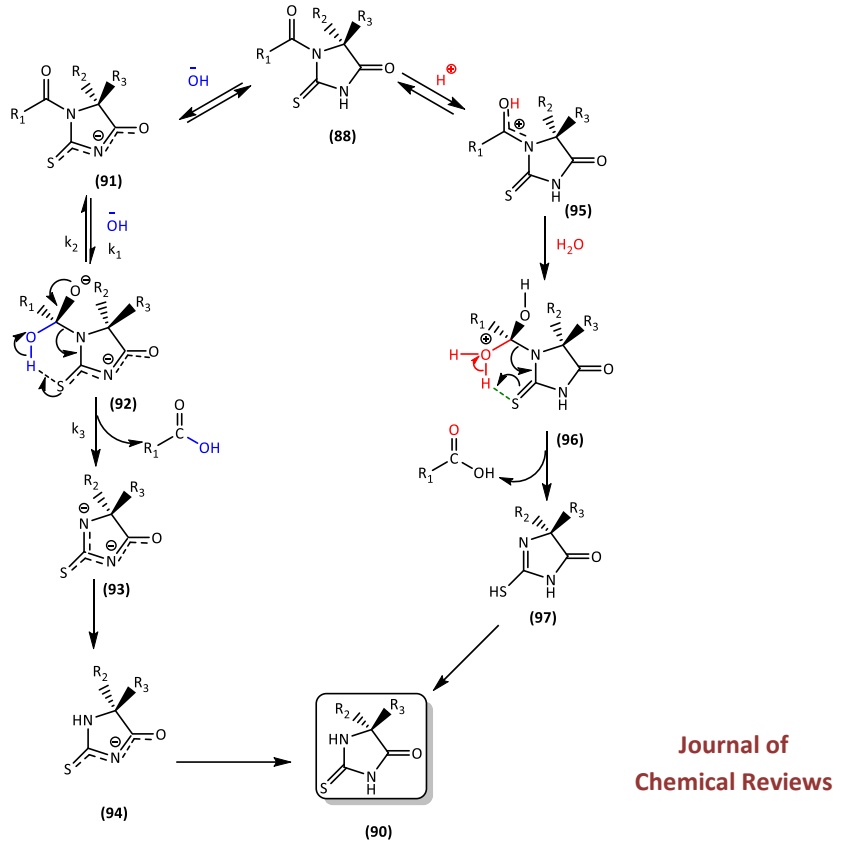

Figure 24. The mechanism of base and acid-catalyzed hydrolysis of 5-substituted 1-acyl-2-thiohydantoins [67,68].

Racemization

Racemization plays significant roles in biological systems, particularly in drug development. Various drug molecules are optically active by having one or several chirality elements (i.e., centers, axes or planes of chirality, and generally helicity) [70]. Generally, A wide spectrum of drugs possesses their biological activity by having chiral centers. Only one of the two enantiomers is pharmaceutically active in most cases while the other is inactive or toxic [71, 72]. 5-Substituted 2-thiohydantoins are of pharmaceutical interest due to their possess a stereogenic center at the C-5 position, which leads to racemization of the molecule in the presence of a buffer or a base. In 1996, Cabrera and coworkers determined the enantiomerisation of phenylthiohydantoin-phenylalanine (PTH, 98) and separated the enantiomers by chiral column HPLC eluted by methanol-triethylammonium acetate buffer at pH 4.1 (2:98, v/v, elution rate: 0.8 mL/min) at three different temperatures (25, 30 and 35) [73]. Toussaint et al. reported the synthesis of tetrahydroisoquinoline thiohydantoin (9) as a racemic mixture separated by a chiral HPLC at 20 oC using a mixture of n-hexane-iPOH (90:1, v/v, 1 ml/min) [74]. The racemization rate of this compound has been investigated in an ethanolic solutions-phosphate buffer (75/25, v/v) pH 2 in the presence of acid or base as catalysts. The complete racemization of this compound, which increased linearly by the increase of buffer concentration and temperature needs less than one hour to occur [75]. The asymmetric organocatalysts of thiohydantoins with pyrrolidine moiety (100) that catalyze Michael addition and aldol reactions and have a chiral center at position C-5 have been synthesised and used in the preparation of several organic compounds [49,76].

In 2019, Lee et al. reported the isolation of two thiohydantoins derivatives (11 and 12) (Figure 25) from horseradish roots by chiral HPLC as racemic mixtures. Compound (11) was eluted

with hexane-iPrOH (98:2, v/v, 1 ml/min) as two enantiomers: 101a (8R) at tR: 43.3 min and 101b (8S) at tR :45.2 min. Compound (102) was also eluted as two enantiomers with hexane-iPOH (96:4, v/v, 1ml/min) afforded 102a (8S) at 24.4 min and 102b (8R) at 26.7 min [77].

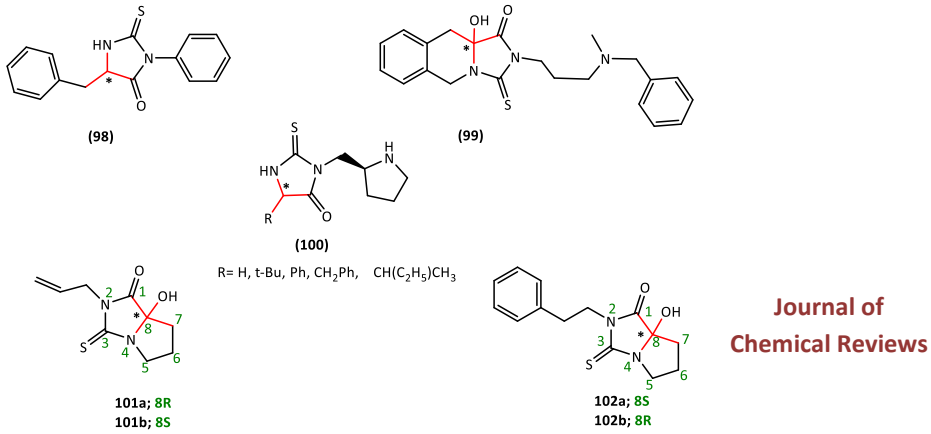

Figure 25. Thiohydantoins compounds with stereogenic center

More recent, Uemura et al. reported the synthesis of thiohydantoins derivatives bearing two chiral centers as a mixture of two diastereomers (11). The authors observed that the epimerization of these diastereomers occurred via ring-opening and closing reactions (Figure 26). Diastereomer 103a was more thermodynamically preferably than 103b. [78,79].

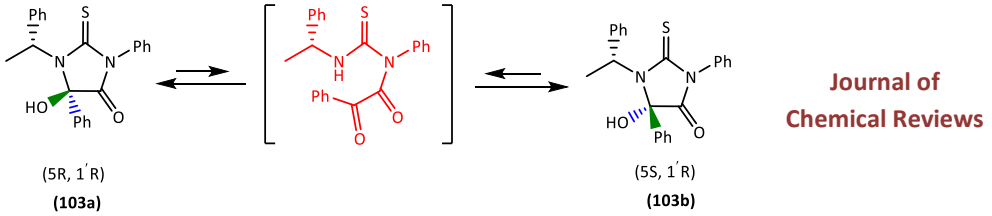

Figure 26. Epimerization of thiohydantoins diastereomers in solution by ring-opening and closing reactions

Condensation and S-alkylation reactions of 2-thiohydantoins

Thiohydantoins have been used in the synthesis of biologically active compounds as intermediates. The most common reaction is condensation with aldehydes at position C-5 [80,81]. More recently, Marton et al. [82] prepared 5-(arylmethylene)-2-thiohydantoin derivatives (15) by condensed 2-thiohydantoin (14) with various aromatic aldehydes (106) in the presence of ethanolamine (Figure 27).

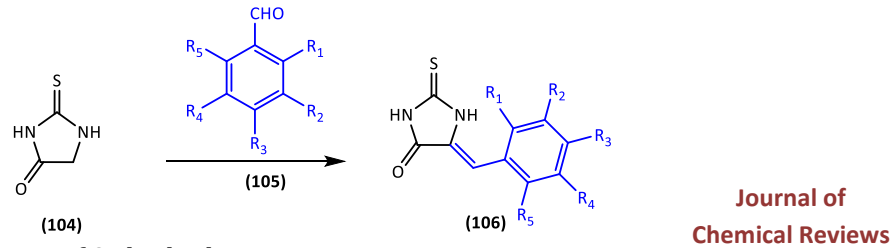

Figure 27. The condensation of 2-thiohydantoin

Alkylation of sulfur atom connected to C-2 in 2-thiohydantoin core in compound (17) can be selectively performed by treatment with methyl iodide in the presence of potassium carbonate afforded S-alkylated 2-thiohydantoin (18) (Figure 28). Condensation of the alkylated compound at C-5 position was performed by dimethylformamide diethyl acetal (DMF-DEA) under microwave assistance produced compound (19) [83].

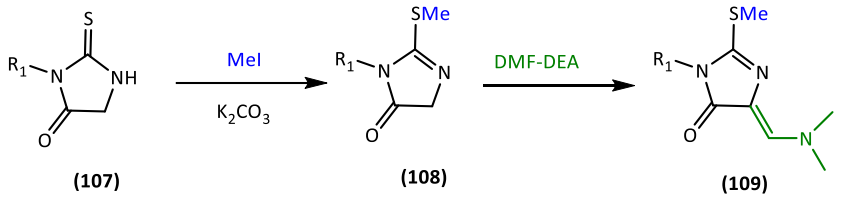

Figure 28. Reactions of 2-thiohydantoins.

N-substitution reaction of thiohydantoin has been reported by Jung et al. [18] where they added various 4-halo aromatic compounds (10) to thiohydantoin derivatives (11) produced N-substituted 2-thiohydantoin derivatives (12) (Figure 29).

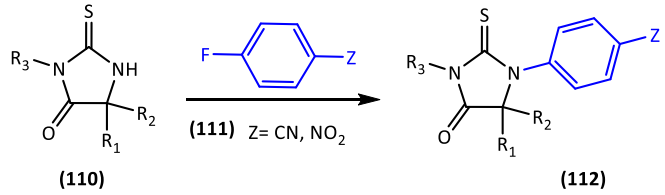

Figure 29. Substitution reaction of 2-thiohydantoins at position N-1

Marine natural product Leucettamine B (15) was synthesised in three steps with an overall yield of 93% starting from commercially available methyl glycinate hydrochloride and methyl isothiocyanate produced 3-methyl-2-thiohydantoin (13) (Figure 30). Following, a condensation reaction at position 5 in thiohydantoin ring with piperonal gave compound (14). The last step was treated with ammonia in the presence of tert-butyl hydroperoxide [84].

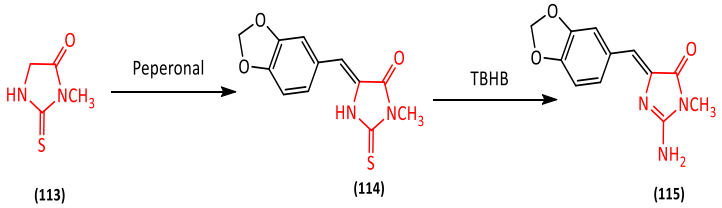

Figure 30. Synthesis of Leucettamine B starting from 2-thiohydantoin analogue

Marine alkaloid from the sponge Agelas dispar anti-histaminic dispacamide (12) has been synthesised simply by coupling reaction between 2-thiohydantoin precursor (16) and compound (11) followed by alkylation of the thiol group in the 5-substituted 2-thiohydantoin (11) produced the intermediate (11), which then treated with ammonia to afford the desired product (120) (Figure 31) [85].

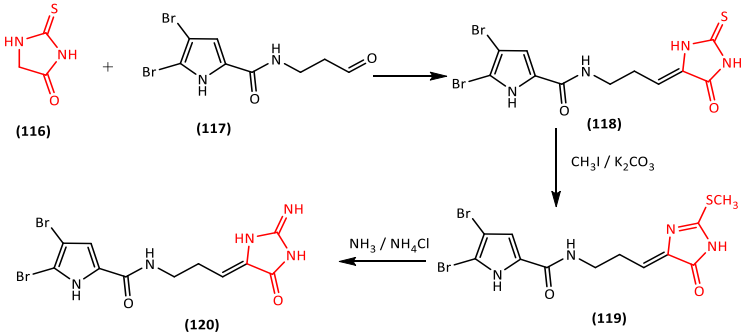

Figure 31. Synthesis of dispacamide from 2-thiohydantoin

Thiohydantoins in protein sequencing

Sequential degradation of amino acids in the protein chain is a well-established procedure for determining protein primary structure. Schlack and Kumpf [62] introduced a method for protein sequencing based on Johnson reaction [1] by labelling the C-terminal end of protein or peptide as acylthiohydantoins (12) using thiocyanate (for example; ammonium thiocyanate) and acetic anhydride to activate the terminal carboxylic acid of protein or peptide (12) followed by cleavage of thiohydantoin in acidic conditions, which then hydrolyzed in alkaline medium afforded the corresponding amino acid (Figure 32). The liberated thiohydantoin derivative (123) can then be detected by HPLC and the remained peptide (12) can be undergoing further degradation. However, this procedure is not suitable for all amino acids, as some cannot form the corresponding 2-thiohydantoins derivatives easily such as glutamine, glutamate, threonine, asparagine, arginine, lysine, and proline [86–89].

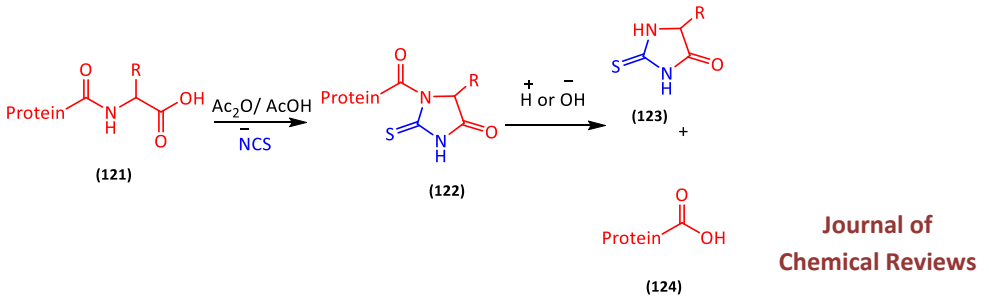

Figure 32. Thiocyanate degradation of protein (incorporating the Schlack and Kumpf's reaction)

Developments of this procedure have been reported by several authors to overcome all existing limitations and to be suitable for any length of the protein chain. Cromwell and Stark [4] used a homogeneous solution from hexafluoroacetone and acetic acid as a solvent instead of a heterogeneous mixture to achieve sequential degradation of amino acids without break down internal peptide bonds of protein. New chemical approaches have been successfully applied successfully on the amino acids that have sensitive groups in the side chain before routine sequencing of the peptide (15) by alkylated peptidyl thiohydantoin intermediate (126 TH) to be easier to leaf. The S-alkylated intermediate (127 ATH) was then cleaved by thiocyanate under acidic conditions afforded (19 ATH) and truncated (128 TH), which simultaneously formed from the adjacent amino acid residue to the ATH (Figure 33) [90–92].

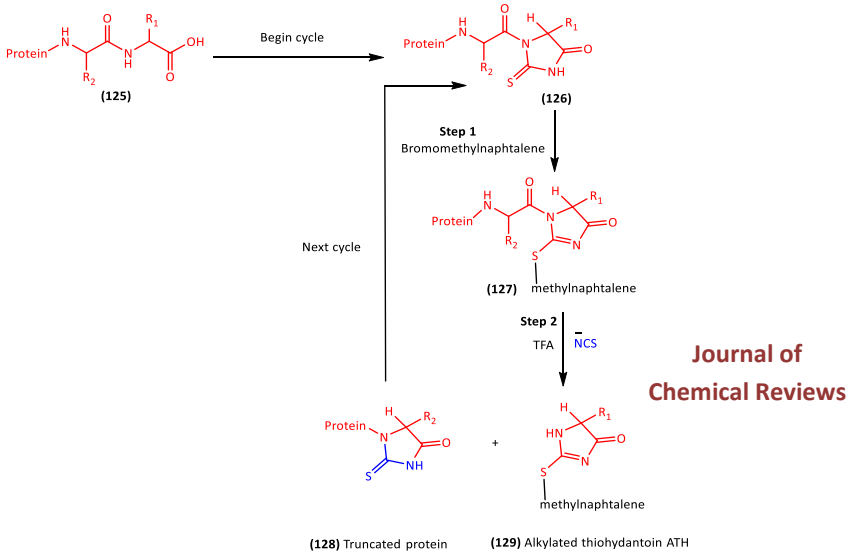

Figure 33. Alkylation procedure for C-terminal protein sequence analysis [90]

In 1950, Edman [91] established an efficient procedure to determine the protein primary structure, which included the reaction of phenyl isothiocyanate at the N-terminal of peptide or protein (13) in alkaline medium afforded phenylthiocarbamyl PTC (11) with subsequent degradation to thiazolinone derivative (12), which then converted in acidic conditions to 2-thiohydantoin derivative (14) (Figure 34).

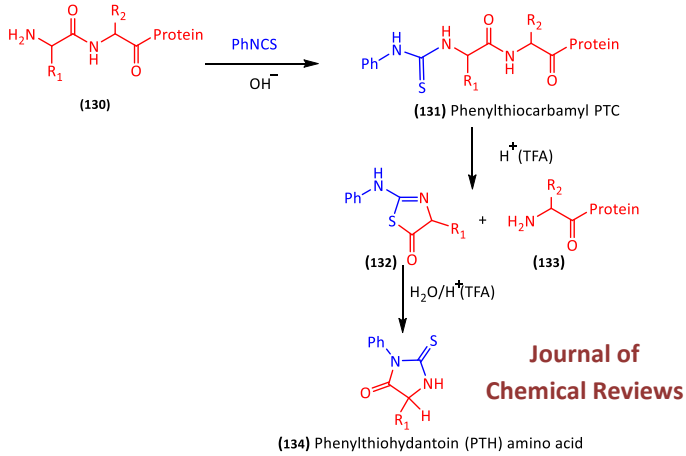

Figure 34. Edman degradation of N-terminal sequencing protein [93–95]

Developments on C-terminal protein sequencing procedure by activating carboxyl and protecting amino groups in amino acids (15) with acetyl chloride (16) before they treated with trimethylsilyl isothiocyanate afforded N-acetyl thiohydantoins (17), which then deacetylated to (18) as reference standards have been reported (Figure 35) [14]. These modifications on the method avoided the limitation of the original such as the reactivity of side chains and difficult solubility of some amino acids as well as the low stability of the thiohydantoin ring. After that, it became suitable to apply on all 20 amino acids. Moreover, the high reactivity of acetyl chloride is the main reason for its choice as activating reagent rather than using acetic anhydride, which led to higher yields from the products than that from a classical method.

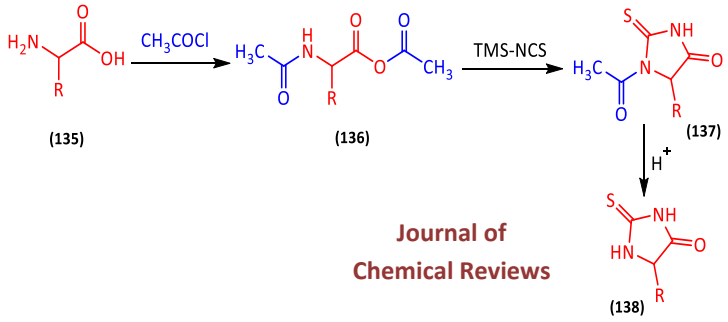

Figure 35. formation of amino acid thiohydantoins from free amino acids activated by acetyl chloride

Conclusion

Hydrolysis, racemization, synthesis, and reactions of 2-thiohydantoins have been summarized in terms of their participation in the drug discovery concept and approaches. Most suggested applications of their scaffolds in medicinal chemistry are also highlighted. The presented data showed that the diversity of chemical synthesis of thiohydantoins has benefited many drug discovery projects. The chemical structure of these molecules possesses two main variable positions N_3 and C_5, where most of their derivatives could be synthesized. The organic synthesis of 2-thiohydantoins has been achieved by various feasible approaches and their reactions are valuable in the synthesis of bioactive molecules. Moreover, the hydrolysis

catalyzed reaction of acyl-substituted 2-thiohydantoins, which causes deacetylation of the acyl group in position N3 plays a critical role in protein sequencing. The attractive feature of their structure is the stereogenic center at position C-5, which gives them biological importance. Given what was previously mentioned in this review, we presented some recent applications of thiohydantoin containing compounds that have pharmacological profiles.

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Conflict of interest

The authors declare no conflict of interest.

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