Section II

Chapter 2: Review of Literature

2. Review of Literature

It was found that 'small molecule' libraries based upon heterocyclic templates are more likely to produce leads with the necessary physicochemical and pharmacokinetic properties required to make a drug.[55-58] This is more relevant while seeking molecules targeting the central nervous system (CNS), where high polarity of molecules would prevent their entry through the blood-brain barrier. Quinazolinone is one such fused heterocycle ring of interest because of its wide range of biological activity. Several quinazolinone alkaloids are known for their wide variety of biological responses. This fact motivated the scientists to prepare and evaluate biologically a number of quinazolinone derivatives and the work still continues.

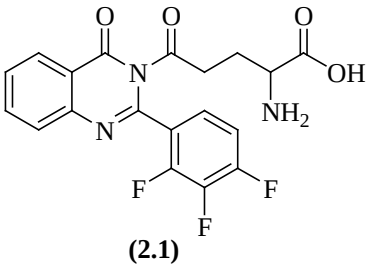
Quinazolinone is a class that is one of the most frequently encountered heterocycles in medicinal chemistry with wide applications. The scaffold is of considerable interest because of a diverse range of their biological properties, for example, antimicrobial, antioxidant, anti-cancer, diuretic, analgesic, anti-inflammatory, anticonvulsant, antiviral and antihypertensive activities.[59,60] The molecules were further explored[61] for CNS and their cognitive functioning, which opened the way for researchers to develop quinazolinones for various neurodegenerative diseases like Alzheimer's, and Huntington's. Several quinazolinone derivatives either synthesized or derived from plant alkaloids have shown vast biological applications. In this chapter we have briefly discussed representative quinazolinone moieties and their role in various diseases/disorders. Focus has been given to the role of quinazolinone derivatives in the treatment of Alzheimer's disease.

2.1 Biological importance of quinazolinones

2.1.1 Quinazolinones as antimicrobial and antioxidant agents

Prashanth and Revanasiddappa synthesized 2,3-disubstituted quinazolinone derivatives and evaluated them for *in vitro* antibacterial activity by disk diffusion method against different types of bacteria using streptomycin as the standard antibacterial agent.[5] All of the synthesized compounds showed significant inhibitory activity against the tested bacterial strains and exhibited moderate to good activity. From the data they concluded that compounds with halogen substituted rings exhibited better antibacterial activity e.g.

compound (**2.1** MIC 8.6 μg/ml). They reported that α-amino carboxylic function in the synthesized compounds helped to improve activity because of its high polarity which would help to penetrate the molecules through the lipid membrane and inhibit the growth of the microorganisms.



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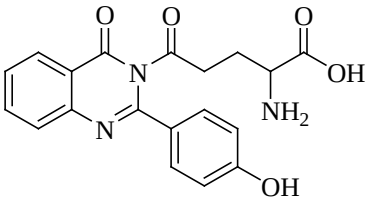

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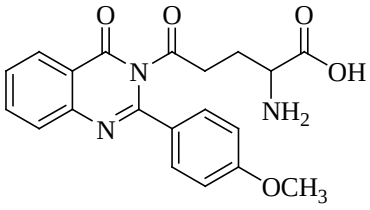

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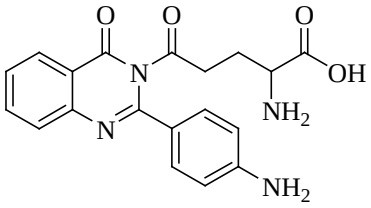

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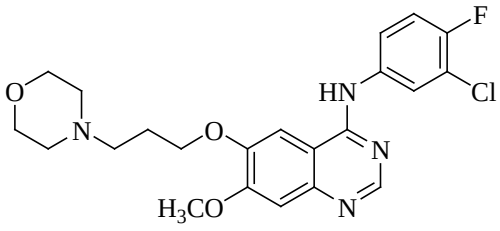
Prashanth and Revanasiddappa also performed antioxidant activity of the synthesized compounds. They performed *in vitro* assays namely, inhibition of DPPH radical, superoxide radical scavenging, hydroxyl radical scavenging, and NO radical scavenging activities to assess the antioxidant properties of the synthesized compounds, in comparison to the standards, such as ascorbic acid and BHA. Compound (**2.2** IC_{50} 14.3 μg/ml) having hydroxyl group at *p*-position in the aromatic ring shows good activity due to high electron releasing property of hydroxyl group which activates the aromatic ring. Compound (**2.3** IC_{50} 18.4 μg/ml) with electron donating methoxy group at para position showed a better DPPH radical scavenging activity. They concluded that compounds (**2.2, 2.3** and **2.4**) were the most efficacious antioxidants due to the presence of electron donating OCH_3, NH_2, and OH groups on the phenyl ring.

2.1.2 Quinazolines and Quinazolinone as anti-cancer agents

Barker *et al*. synthesised a series of 4-anilinoquinazoline compounds to target cancer as the epidermal growth factor receptor tyrosine kinase inhibitors.[62] They identified **2.5**

having good oral bioavailability and inhibited the growth of a broad range of human solid tumour xenografts in a dose-dependent manner with marked regression seen in some tumours. All of the synthesized compounds in the series showed good anti-cancer activity while **2.5** showed better *in vivo* results compared to *in vitro* for the anti-cancer activity with a long half-life in humans compatible with once a day oral dosing. Extensive Phase I clinical trials have shown biomarker evidence for inhibition of the EGFR signal transduction pathway and antitumour activity for the compound. There are other reports published by Gawad *et al*.[83] for the 2,3-disubstituted quinazolinones as broad-spectrum antitumors showing effectiveness toward numerous cell lines belonging to different tumor subpanels and **2.6** was found to be the most potent compound.



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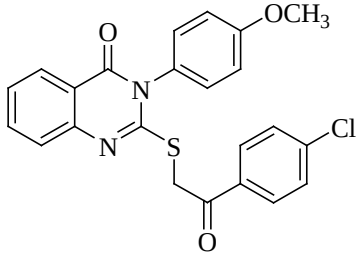

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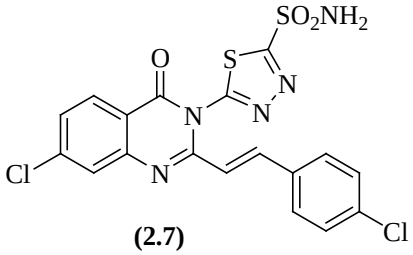
2.1.3 Quinazolinones as diuretic agent

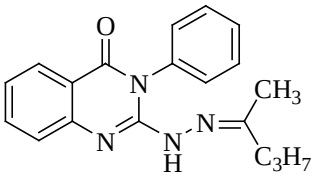
Marrouf *et al*. reported a series of quinazolinone derivatives containing either a thiazole or a 1,3,4-thiadiazole moiety and evaluated them for their diuretic activity.[64] Since vinyl group at 2 position helps in good diuretic activity, 2-(*p*-chlorophenyl)vinyl group was found to improve diuretic action. Among all of the synthesized compounds, 2-[2-(4-chlorophenyl)vinyl]-7-chloro-3-(2-sulfamoyl-1,3,4-thiadiazol-5-yl)quinazolin-4(3*H*)-one (**2.7**) exhibited significant diuretic activity.



(2.7)

2.1.4 Quinazolinones as analgesics and anti-inflammatory agents

In another study carried out by Alagarsamy *et al*. a number of quinazolinone derivatives were synthesized and evaluated for analgesic and anti-inflammatory activities.[65] It was reported earlier that the presence of alkyl groups exhibited higher analgesic and anti-inflammatory activities over the aryl groups at the N-3 position.[66,67] Aryl substitution at N-3 and 2-pentylidene-hydrazino and various other hydrazino functions at 2-position were introduced to increase hydrophilicity of the molecules which enhanced the analgesic and anti-inflammatory activities. Among the synthesized compounds, compounds (**2.8** and **2.9**) showed potent analgesic and anti-inflamatory activities.



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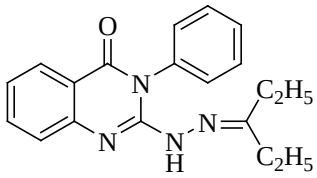

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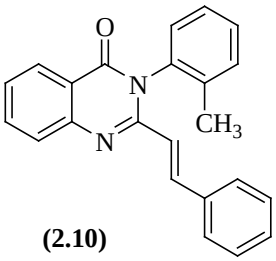
2.1.5 Quinazolinones as anticonvulsant agents

Some reports available in literature revealed that substituted quinazolin-4(3*H*)-one exhibited anticonvulsant activity. Boltze *et. al.* have worked on the same line and reported the synthesis of 2-(2-arylethenyl)-3-*o*-tolyl-4(3*H*)quinazolinone derivatives.[68] Majority of the compounds showed mild to moderate anticonvulsant activity but compounds (**2.10** and **2.11**) showed very good anticonvulsant activity. There are a number of other reports on quinazolinone derivatives with some modifications in the substituents at 2 and 3 positions claiming for moderate to good anticonvulsant activity.[69-72]



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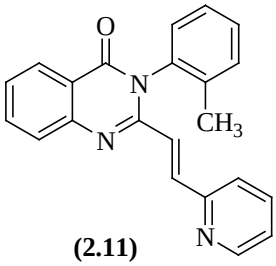


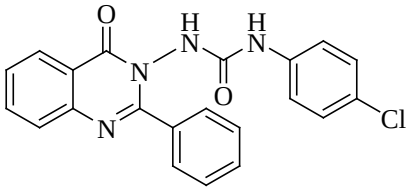
(2.11)

In another publication Kashawa *et al*. have synthesized several new 1-(4-substituted phenyl)-3-(4-oxo-2-phenyl/ethyl-4*H*-quinazolin-3-yl)ureas and screened them for CNS depressant activity by maximal electroshock induced seizures (MES) and subcutaneous

pentylenetetrazole (scPTZ) induced seizure models in mice.[73] In their studies, they found that majority of the compounds with para substituted phenyl ureas at position 3 were active in the MES screen as well as in the scPTZ screen. Compound (**2.12**) was the most active compound among them.

2.1.6 Quinazolinones as antiviral agents

Xingwen *et al*. developed simple and general method for the synthesis of quinazolinone derivatives and evaluated the synthesized compounds for their antiviral activity.[74] Majority of the compounds were found to possess moderate to good antiviral activity but activity of compound (**2.13**) was ascertained by semi-quantitative PCR (polymerase chain reaction) and real time PCR assays against tobacco mosaic virus (TMV). They also suggested that **2.13** possessed antiviral activity by induction and up-regulation of PR-1a and PR-5 genes thereby inhibiting proliferation and movement of the virus, by enhancing activity of some defensive enzymes.



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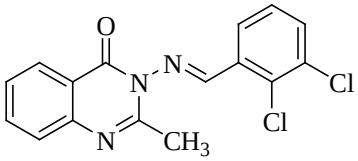
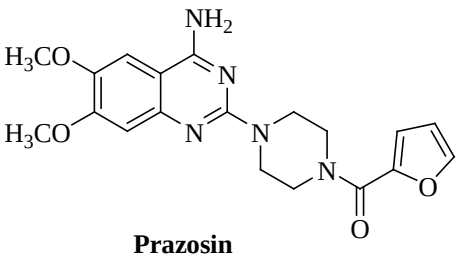


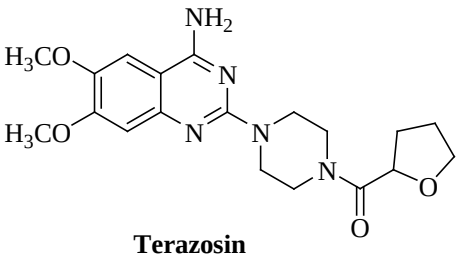
(2.13)

2.1.7 Quinazolines as antihypertensive agents

Quinazolines are very well established for their antihypertensive properties, as many of the antihypertensive drugs contain quinazoline moiety e.g. prazosin (**2.14**), terazosin (**2.15**), doxazosin, bunazosin, tiodazosin, trimazosin and alfuzosin. Harsha *et al*. reported some quinazolinones that showed moderate antihypertensive properties.[75] Researchers are still working on the development of potent and effective quinazolinone derivatives as antihypertensive agents.[76]



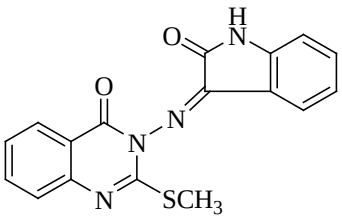
Prazosin
(2.14)



Terazosin
(2.15)

2.1.8 Quinazolinones as anti-HIV agents

In a report Pandeya *et al*. claimed anti-HIV properties of quinazolinone derivatives.[77] They have synthesized 3-amino-2-methylmercaptoquinazolin-4(3*H*)-ones from anthranilic acid. The synthesized compounds were evaluated for anti-HIV activity against HIV-1 III B. in MT-4 cells and found that compound (**2.16**) possessed good activity.



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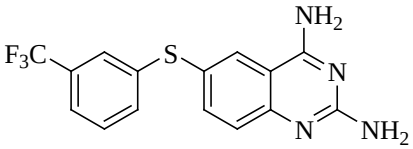


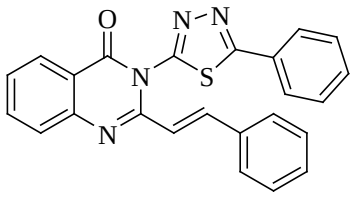
(2.17)

2.1.9 Quinazolines as antimalarial agents

Werbel *et al*. synthesized[78] a variety of analogs of 6-substituted quinazolines with antimalarial properties wherein the 4-amino group was replaced by hydrazine and hydroxyamino moieties and found that such changes markedly reduced the antimalarial properties of the compounds. The compound (**2.17**) was tested against a normal drug-sensitive strain of *Plasmodium berghei* in mice by the parenteral route.[78]

2.1.10 Quinazolinones as CNS depressant agents

Quinazolinones also showed some CNS activity. Jatav *et al*. synthesized a series of novel 3-(5-substituted phenyl-1,3,4-thiadiazol-2-yl)-2-styrylquinazoline-4(3*H*)-ones and evaluated them for CNS depressant activity using forced swim pool method.[79] They found that compound (**2.18**) was the most active for CNS depressant activity.



(2.18)

2.2 Role of quinazolines and quinazolinones in the Alzheimer's disease

A thorough literature review shows that neuritic amyloid plaques and neurofibrillary tangles comprising of hyperphosphorylated tau proteins represent the core neuropathologic features of AD.[80,81] This neurodegenerative disorder is often characterized by a low concentration of acetylcholine (ACh) in hippocampus and cortex.[82] The disease is accompanied by dysfunctions in the system of cholinergic neurotransmission of the central nervous system (CNS).[83] In the CNS, the cholinergic system regulates memory and learning processes, in which two enzymes degrade ACh: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).[84] These two enzymes have been identified as potential targets in the treatment of AD, myasthenia gravis, glaucoma, and in the recovery of victims of nerve gas exposure.[85]

AChE is a key component of cholinergic brain synapses and neuromuscular junctions. The main role of this enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine.[86] BuChE is produced in the liver and enriched in the circulation. The exact physiological role of BuChE is still elusive, but it is generally viewed as a backup for the homologous AChE.[87,88] It is noteworthy that AChE activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10–15% of the normal value, while BuChE activity remains unchanged or even increases by 20%. Therefore, a large pool of BuChE is available in glia neurons and neuritic plaques.[89] It may not be an advantage for a cholinesterase inhibitor (ChEI) to be selective for AChE; on the contrary, a good balance between AChE and Bu-ChE may result in a higher efficacy.

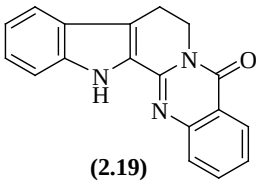
It is reported that the genesis of amyloid protein plaques associated with AD is connected to modifications of both AChE and BuChE, since the plaque is significantly decreased in AD patients using ChE inhibitors (ChEIs).[90-93] Consequently, it is not surprising

that ChEIs have shown more promising results in the treatment of AD than any other strategy explored.[94-97]

The catalytic active site of AChE was found to be located at the bottom of a narrow gorge, and consists of two sub sites, a negatively charged or 'anionic' site, and an esteratic site, containing the actual catalytic residues, also called the catalytic triad.[98] BuChE possesses a larger void at the active site gorge,[99] AChE contains a second 'anionic' site, which became known as the 'peripheral anionic' site (PAS) that lies around 14Å away from the active site.[100]

Recent studies have identified that AChE could also play a key role in accelerating senile amyloid β-peptide (Aβ) plaques deposition.[101] It is likely that AChE interacted with Aβ and promoted amyloid fibril formation through a pool of amino acids located in the proximity of peripheral anionic site (PAS).[102]

According to cholinergic hypothesis, small heterocyclic molecules are good enough to maintain the level of acetylcholine in brain. Research into the cholinergic hypothesis has led to the development of several fused and non-fused ring systems as cholinesterase inhibitors (ChEIs). There are numerous biologically active molecules (choline esterase inhibitors) whose framework includes a six-membered ring containing two nitrogen atoms fused to a phenyl ring. Most of these molecules are based on the **quinazoline** or **quinazolinone** framework.



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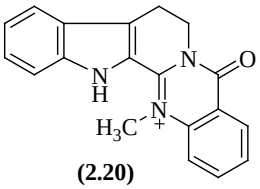

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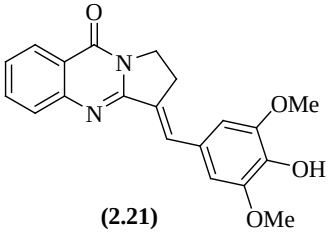

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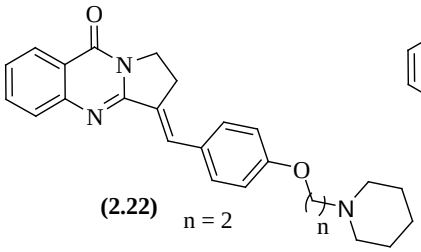
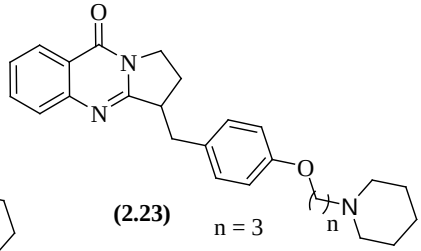


(2.22) n = 2

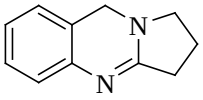


(2.23) n = 3

Rutaecarpine (**2.19**), a major quinazolinocarboline alkaloid isolated from *Evodia rutaecarpaas* and some of its synthetic derivatives including dehydroevodiamine (DHED)

(**2.20**) have been described as choline esterase inhibitors.[103] The alkaloid isaindigotone (**2.21**) isolated from the root of *Isatis indigotica* Fort, a biennial herbaceous plant widely present in China[104] and its synthetic derivatives (**2.22, 2.23**)[105] have been reported as ChEI.

Another alkaloid deoxyvasicine (**2.24**) has been described as an inhibitor of AChE.[106,107] Decker *et al*[108] described chemically related tri- and tetracyclic nitrogen bridgehead compounds (**2.25-2.27**), as moderate or strong inhibitors of AChE and BuChE, showing different selectivity profiles. Decker *et al*[109] also studied the SAR of the 7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-ylidene derivatives (**2.27** and congeners). SAR studies have shown that as the distance between the phenyl ring and the heterocycle ring was reduced, the selectivity towards the BuChE increased.



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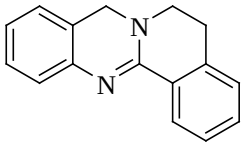

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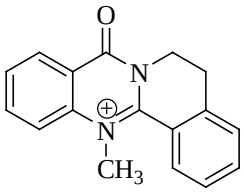

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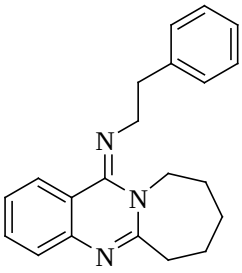

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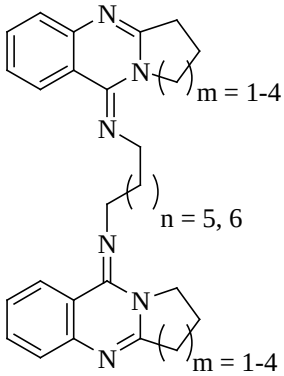
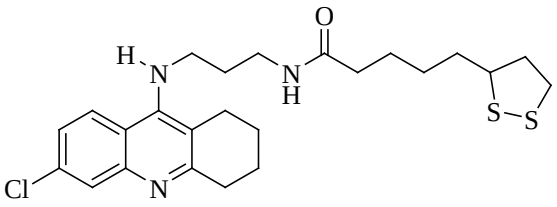
Chen *et al*[109] and Decker *et al*[110] reported a series of bivalent quinazolinimines (**2.28**) as selective and potent BuChE inhibitors.



(2.28)

Several novel strategies have been applied in medicinal chemistry to speed up AD drug development, for example, well-known inhibitors (like tacrine) have been used in so-

called hybrid molecules having two distinct drug molecules covalently connected.[111-114] Additionally, a bivalency approach (formerly applied to GPCR ligands, like opioid agonists and dopamine antagonists)[115,116] has been applied to AChE inhibitors (i.e., to connect two inhibitor molecules covalently by a long hydrocarbon spacer).[117]



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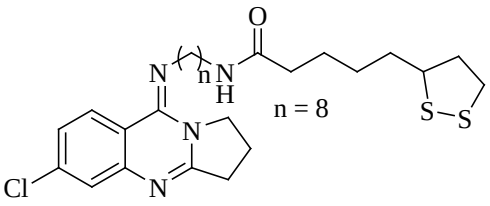

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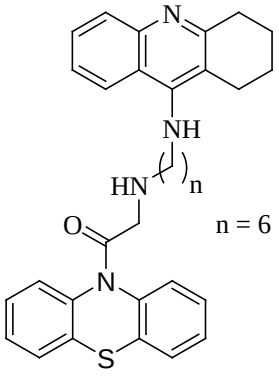
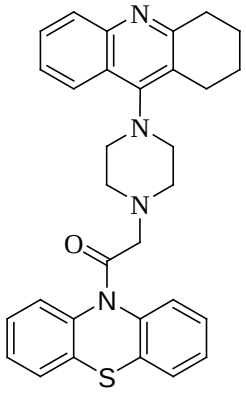
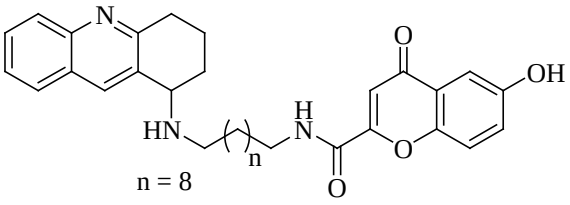


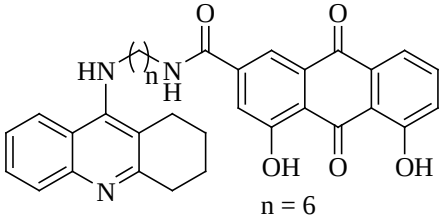
(2.31)



(2.32)



(2.33)



(2.34)

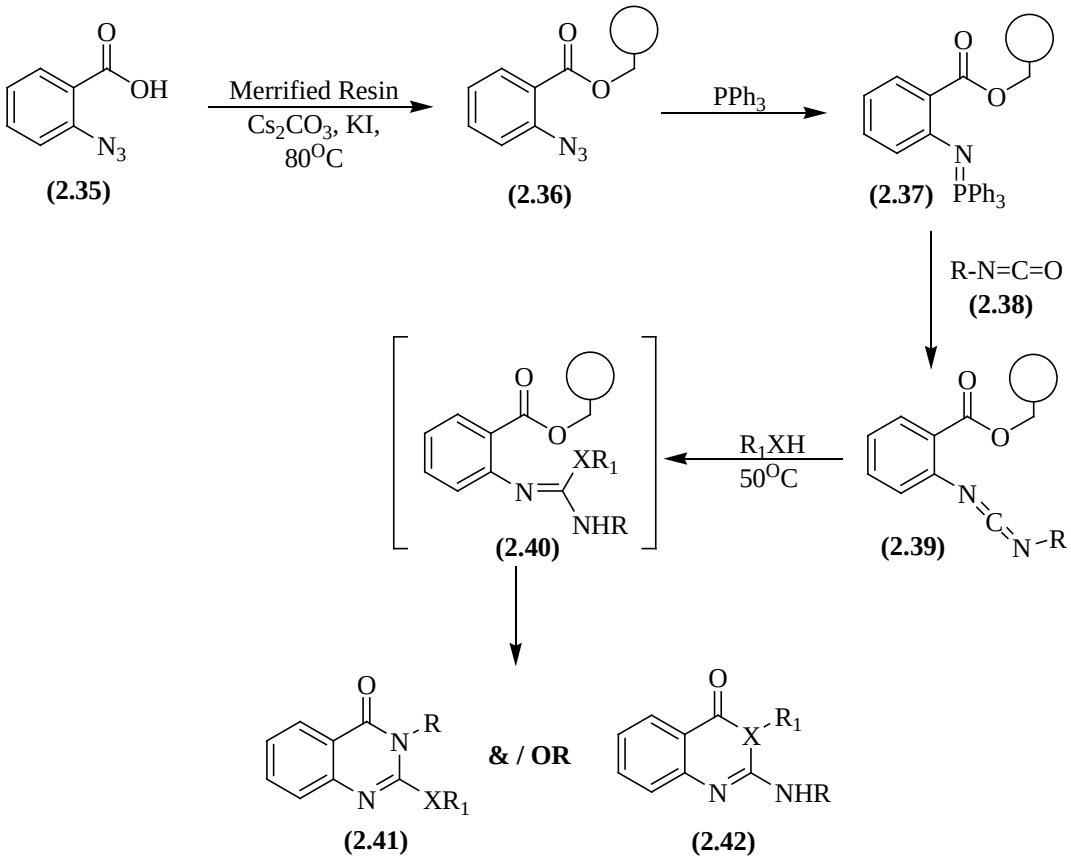
Some hybrids have been reported as active compounds which contain tacrine moiety and PAS binding moiety linked via suitable linkers. Michela *et al*[118] reported Tacrine connected to lipoic acid hybrids (**2.29**) whose biological profiles were markedly improved relative to those of the prototypes tacrine and lipoic acid. They were also found to inhibit the AChE induced β-amyloid aggregation indicating their interactions with the peripheral anionic site (PAS), which accelerated amyloid fibril formation.[119,120] Decker *et al*[121] reported a series of *N*-[6-chloro-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1*H*)-ylidene]alkane-α,ω-diamines

coupl-ed with lipoic acid hybrids, among which a compound having n=6 (**2.30**) was found as the most potent one. It was able to inhibit β-amyloid fibril formation, which was proved by kinetic measurements and showed mixed type of inhibition by interacting with both, the active site and the PAS. Hui *et al*[122] reported a series of tacrine-phenothiazine hybrids (**2.31, 2.32**) as multitarget drugs for Alzheimer's disease which inhibited not only the degradation of the neurotransmitter ACh but also the aggregation of the beta-amyloid protein and phosphorylation of the Tau protein. Tacrine–4-oxo-4*H*-chromene hybrids (**2.33**) have been designed as multifunctional agents capable of inhibiting ChE and β-secretase[123] Li *et al*[124] reported tacrine–rhein hybrids (**2.34**) as multifunctional agents to inhibit CAS (Catalytic Anionic Site) as well as PAS.

2.3 Synthetic approaches for Quinazolinones

Various synthetic strategies have been described in the literature for the synthesis of quinazolinones from anthranilamide and related compounds. The first one achieves ring closure through the amide bond[125] and the second uses carbodiimide formation on amino nitrogen to affect ring closure between the amide nitrogen with the carbon spacer.[126] Other strategies involve the reaction between isatoic anhydride and thiourea derivatives to cause ring opening[127,128] and the use of orthoformates to react with both the nitrogen atoms for the generation of quinazolinones.[129] Some groups have utilized the quinazolinone scaffold in combinatorial synthesis.[130,131] A few of the representative syntheses are described here.

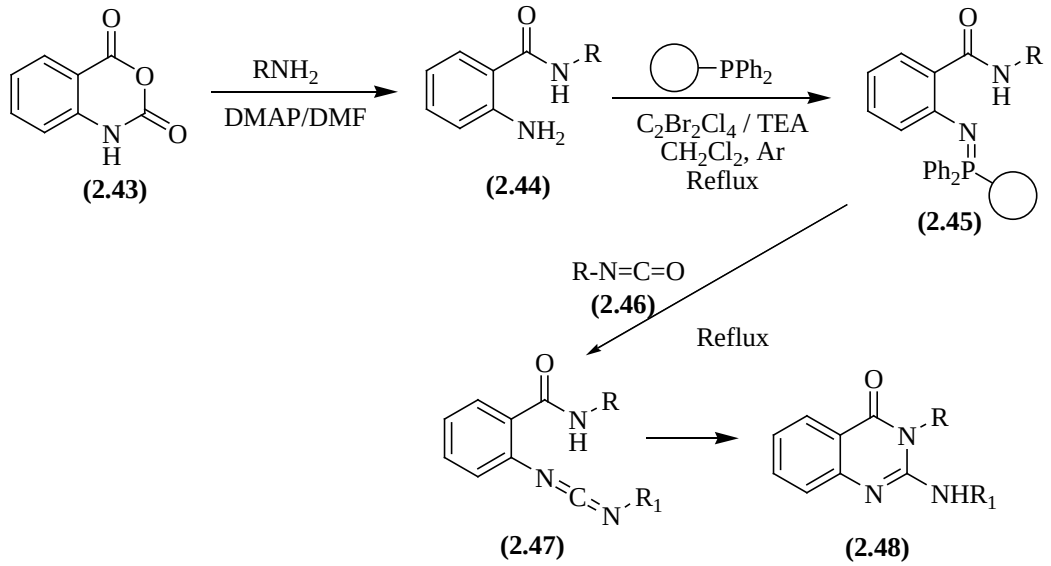
Quinazolinones were synthesized by Villalgordo *et al*. using an aza Wittig mediated annulation strategy (**Scheme 2.1**).[125] After alkylative esterification of the o-azido benzoic acid (**2.35**) with Merrifield resin, the product (**2.36**) was treated with triphenylphosphine to yield the iminophosphorane (**2.37)**. Division of the resin beads and subsequent aza Wittig reaction with different isocyanates (**2.38**) afforded a variety of carbodiimides (**2.39**). Further division of the resin and treatment with various nucleophiles yielded (**2.40**), which underwent intramolecular cyclization and simultaneous cleavage from resin to form the quinazolinones (**2.41** and **2.42**). Unfortunately, the control over the ratio of products (**2.41** and **2.42**) decreases to 1:1 when sterically less hindered groups are used at R_1.



Scheme 2.1 Quinazolinone synthesis by Villalgordo *et al.*[125]

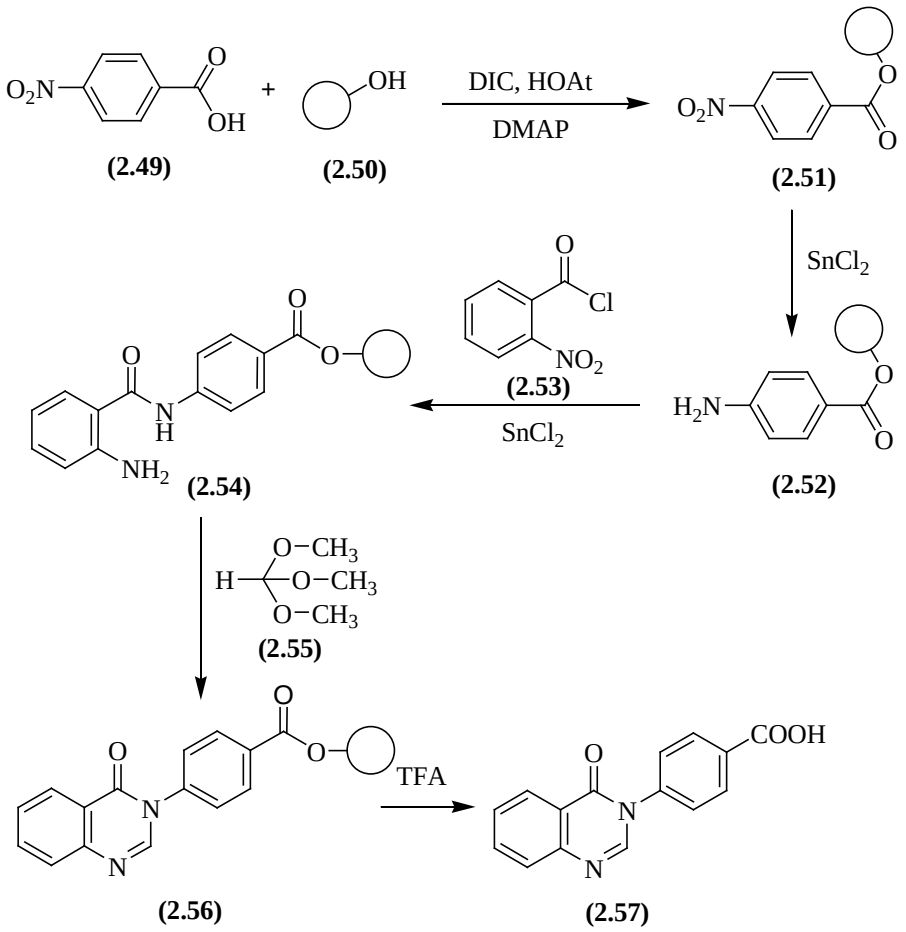
A similar synthesis was reported by Zhang and co-workers.[126] As can be seen in (**Scheme 2.2**), it is very similar to that described by Villalgordo *et al.*[125] but provides far better control over the products. Treatment of isatoic anhydride (**2.43**) with substituted primary amines in *N*,*N*-dimethylformamide (DMF) with *N*,*N*-dimethylaminopyridine (DMAP) produced the *N*-substituted benzamides (**2.44**). Using a modified Kirsanov reaction, **2.44** was then treated with polymer-bound triphenylphosphine in the presence of dibromotetrachloroethane and triethylamine in dry dichloromethane at reflux under argon to produce the resin-bound iminophosphorane (**2.45).** Heating of this product with isocyanates (**2.46**) in dry toluene or xylene under argon cleaved the product from resin, producing the carbodiimide intermediate (**2.47**). Intramolecular cyclization then occurred to yield the 4-

quinazolinone (**2.48**) in good yield (68-89%) and purity (61-96%) across seven quinazolinones.



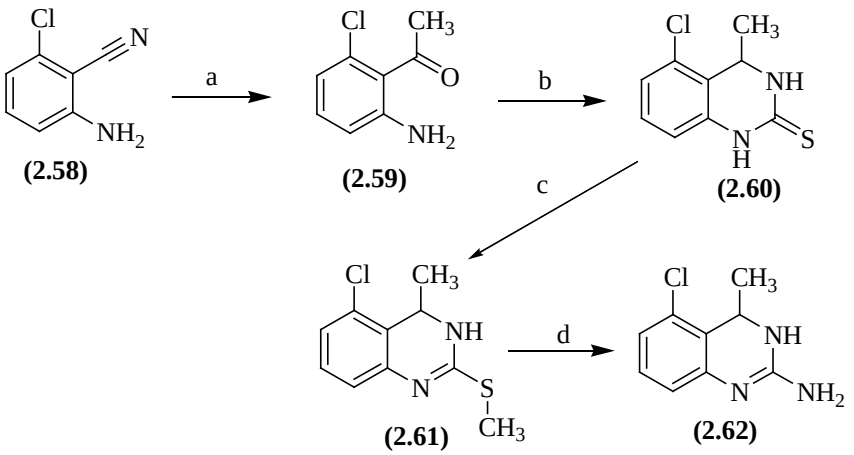
Scheme 2.2 Quinazolinone synthesis by Zhang et al[132]

A different synthesis was developed by Makino *et al*. (**Scheme 2.3**).[129] This procedure utilizes mild acidic conditions and allows the use of compounds sensitive to oxidation. The scheme begins with the attachment of 4-nitrobenzoic acid (**2.49**) to a Synphase Lantern (**2.50**) using 1-hydroxy-7-azabenzotriazole (HOAt), DIC, and DMAP. Treatment of the resultant compound (**2.51**) with tin chloride then produced the amine (**2.52**)**,** which could then be treated with the *o*-nitrobenzoylchloride (**2.53**), and the nitro group reduced with tin chloride, yielding **2.54**. Addition of a trimethyl orthoformate (**2.55**) cyclized the resin bound quinazolinone to yield the product (**2.56**)**,** which was subsequently cleaved from the resin with trifluoroacetic acid to afford quinazolinone (**2.57**). A similar solid phase strategy has also been used by Theoclitou *et al*.[132]



Scheme 2.3 Quinazolinone combinatorial synthesis by Makino *et al*[76]

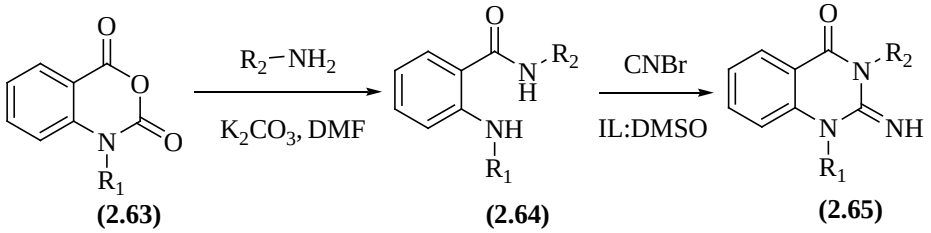
Dihydroquinazolinylamines with an alkyl substituent in the 4-position, such as **2.58**, were obtained as outlined in **Scheme 2.4**.[133] The addition of a Grignard reagent to aminobenzonitrile **(2.59)** and hydrolysis of the resulting imine gave an aminoacetophenone **(2.59)**. This amino-ketone was converted in a one-pot reduction-HSCN sequence to a cyclic thiourea **(2.60)**. Methylation provided isothiourea **(2.61)** as its hydroiodide. Nucleophilic replacement of the methylsulfanyl leaving group by ammonia then gave the target compound[134] **(2.62)**.



Reagents and conditions: (a) MeMgBr,Et_2O, 3 h reflux; then 6 N HCl, 4 h reflux, 67%; (b) $NaBH_4$, EtOH, 65^0C overnight; then KSCN, HCl, 3 h 65^0C, 43%; (c) MeI (3 equiv), acetone, weekend rt, 87%; (d) NH_4OH, H_2O/CHCN, microwave30 min 170^0C, 62%

Scheme 2.4 Representative preparation of a 4-alkyl substituted dihydroquinazolinyllamine

For preparation of the target compound 2-imino-4-quinazolinones (**2.65**), Verma *et al*. reported a method by substituting isatoic anhydride (**2.63**) with suitable primary amines to efficiently synthesize 2-aminobenzamides (**2.64**).[135] Later on, the intermediate (**2.64**) was treated with cyanogen bromide to achieve a ring closer for the synthesis of the desired quinazolinones (**2.65**) (**Scheme 2.5**).



Scheme 2.5. Synthesis of quinazolinone as cyclic guanidines

There are still a large number of synthetic methods reported for the preparation of differently substituted quinazolinones.[136] Due to its wide range of biological properties, chemists are still developing more convenient methods for the synthesis of quinazolinone and fused quinazolinone derivatives.