

Pharmaceutical Chaperones: A Novel Therapeutic Strategy for Protein Misfolding Disorders

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Abstract

Protein folding is an essential biological process that enables proteins to attain their correct three-dimensional structure and perform their physiological functions. Disruptions in protein folding can result in conformational instability, intracellular retention, aggregation, and degradation of proteins, contributing to the development of numerous inherited and acquired diseases. Protein misfolding has been implicated in a wide range of disorders, including lysosomal storage diseases, cystic fibrosis, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, metabolic disorders, and certain cancers. Conventional therapeutic approaches, including enzyme replacement therapy and symptomatic treatments, often provide limited efficacy because they do not directly address the underlying molecular defects responsible for disease progression. Pharmaceutical chaperones have emerged as a promising therapeutic strategy that targets the root cause of many conformational disorders. These small-molecule compounds selectively bind to unstable or misfolded proteins, stabilize their native conformation, facilitate proper folding, enhance intracellular trafficking, and prevent premature degradation by cellular quality-control mechanisms. Consequently, functional protein levels are restored, leading to improved cellular homeostasis and therapeutic outcomes. The clinical success of migalastat in Fabry disease has validated the potential of pharmaceutical chaperone therapy and stimulated extensive research into its application for other protein misfolding disorders. Recent advances in structural biology, computational drug design, artificial intelligence, and proteostasis research have accelerated the identification and optimization of novel pharmaceutical chaperones with enhanced specificity and efficacy. Additionally, combination approaches involving enzyme replacement therapy, gene therapy, and proteostasis regulators are broadening their therapeutic applications. Despite challenges related to mutation specificity, safety, and target selectivity, pharmaceutical chaperones represent an innovative class of precision therapeutics with significant potential for the treatment of diverse conformational diseases and the advancement of modern drug discovery.

Keywords

Pharmaceutical Chaperones; Protein Misfolding; Proteostasis; Migalastat; Precision Medicine.

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1. Introduction

Proteins are essential biological macromolecules that perform structural, catalytic, signaling, and regulatory functions in living organisms. Proper protein folding is crucial for maintaining biological activity, and this process is assisted by endogenous molecular chaperones within the endoplasmic reticulum (ER) and cytoplasm. Genetic mutations, environmental stress, aging, and cellular dysfunction can disrupt protein folding, leading to the accumulation of misfolded proteins and cellular toxicity (1,2).

Protein misfolding is associated with numerous disorders, including lysosomal storage diseases, cystic fibrosis, Fabry disease, Gaucher disease, Alzheimer's disease, Parkinson's disease, and Huntington's disease (3,4). Conventional therapies, such as enzyme replacement and symptomatic treatment, often fail to correct the underlying molecular defects responsible for these conditions (5).

Pharmaceutical chaperones (PCs) are small molecules that selectively bind to unstable or misfolded proteins, stabilizing their native conformation and promoting proper folding, intracellular trafficking, and functional recovery (6). Unlike endogenous molecular chaperones, pharmaceutical chaperones act as exogenous agents capable of rescuing mutant proteins from premature degradation by cellular quality-control systems (7).

The concept of pharmaceutical chaperoning originated from studies demonstrating that small-molecule ligands could stabilize mutant lysosomal enzymes and restore their activity, particularly in Fabry disease through the rescue of α -galactosidase A (8). Since then, pharmaceutical chaperones have been explored for the treatment of several lysosomal, metabolic, and neurodegenerative disorders (9).

These agents function by stabilizing partially folded proteins, reducing aggregation, and facilitating escape from ER-associated degradation pathways, thereby restoring protein function (10). Depending on their mechanism, pharmaceutical chaperones may act as active-site binders, allosteric modulators, or proteostasis regulators (11).

Among their advantages are oral administration, improved tissue penetration, reduced immunogenicity, and the ability to target intracellular proteins. The approval of migalastat for Fabry disease has validated the clinical potential of this therapeutic approach (12,13). Furthermore, advances in structural biology, computational drug design, and artificial intelligence have accelerated the discovery of novel pharmaceutical chaperones for a wide range of conformational disorders (14,15).

This review summarizes the mechanisms, classification, therapeutic applications, advantages, limitations, and future prospects of pharmaceutical chaperones in the management of protein misfolding diseases (16).

2. Protein Folding and Proteostasis

Proteins must attain precise three-dimensional conformations to perform their biological functions. Protein folding is a highly regulated process influenced by amino acid sequence and intracellular conditions such as pH, temperature, and redox state (17). Cellular protein homeostasis, known as proteostasis, is maintained through an integrated network of molecular chaperones, protein degradation pathways, and quality-control mechanisms that regulate protein synthesis, folding, trafficking, and degradation (18).

2.1 Molecular Chaperones and Quality Control

Molecular chaperones are endogenous proteins that assist in proper protein folding and prevent aggregation. Major chaperone families include heat shock proteins (HSP70, HSP90, and HSP60), which recognize unfolded proteins and facilitate their maturation into functional conformations (19). HSP70 primarily assists nascent protein folding, while HSP90 stabilizes signaling proteins and receptors involved in cellular regulation (20).

A significant proportion of newly synthesized proteins undergo folding within the endoplasmic reticulum (ER), where specialized chaperones such as BiP, calnexin, calreticulin, and protein disulfide isomerase ensure correct maturation (21). Proteins that fail to attain proper conformation are retained within the ER and targeted for degradation through the ER-associated degradation (ERAD) pathway (22). Although ERAD protects cells from toxic protein accumulation, it may also eliminate partially functional mutant proteins that could potentially be rescued therapeutically (23).

2.2 Protein Misfolding and Disease

Protein misfolding occurs when proteins fail to achieve or maintain their native structure. Misfolded proteins often aggregate and disrupt normal cellular functions, contributing to disease pathogenesis (24). Protein aggregation is a hallmark of several neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease, where abnormal protein accumulation leads to progressive cellular damage (25).

Similarly, many inherited metabolic disorders arise from mutations that destabilize proteins without completely abolishing their activity. These unstable proteins are frequently retained in the ER and degraded before reaching their functional destinations, resulting in disease manifestations (26).

2.3 Relevance to Pharmaceutical Chaperones

The observation that many mutant proteins retain residual activity has led to the development of pharmaceutical chaperones. These small molecules selectively bind unstable proteins, promote correct folding, prevent premature degradation, and restore intracellular trafficking (30). By rescuing functional

proteins from quality-control pathways, pharmaceutical chaperones offer a targeted therapeutic strategy for a variety of conformational diseases (31).

Thus, understanding protein folding, quality-control systems, and proteostasis mechanisms provides the scientific foundation for the development of pharmaceutical chaperone-based therapies.

3. Pharmaceutical Chaperones: Concept and Development

Pharmaceutical chaperones are small-molecule compounds that selectively bind to unstable or misfolded proteins, stabilizing their native conformation and facilitating proper folding, intracellular trafficking, and function (32,33). Unlike endogenous molecular chaperones, which assist the folding of multiple proteins through ATP-dependent mechanisms, pharmaceutical chaperones act directly on specific target proteins and help prevent their premature degradation by cellular quality-control systems (34).

This therapeutic approach is particularly valuable in conformational diseases where proteins retain residual biological activity but are misfolded and degraded before reaching their site of action. Such disorders include lysosomal storage diseases, metabolic disorders, neurodegenerative diseases, and receptor-associated abnormalities (35).

The concept of pharmaceutical chaperone therapy originated from studies on lysosomal storage disorders during the late 1990s. Researchers observed that certain enzyme inhibitors unexpectedly stabilized mutant lysosomal enzymes and enhanced their intracellular trafficking rather than suppressing their activity (36). A major breakthrough was achieved in Fabry disease, where small molecules were shown to stabilize mutant α -galactosidase A and restore lysosomal function (37). These findings established the foundation for pharmaceutical chaperone therapy and stimulated further investigations into other protein misfolding disorders (38).

Advances in structural biology, molecular pharmacology, and computational drug design have improved understanding of protein–ligand interactions and facilitated the discovery of novel pharmaceutical chaperones targeting enzymes, receptors, ion channels, and transport proteins (39,40). Unlike molecular chaperones, pharmaceutical chaperones exhibit greater target specificity and can be tailored to correct disease-associated folding defects, making them attractive candidates for precision medicine (41–43).

Modern discovery approaches now utilize structure-based drug design, high-throughput screening, and virtual screening, artificial intelligence, and proteomic analyses to identify compounds capable of stabilizing mutant proteins (44, 45). In addition, the development of proteostasis regulators has expanded therapeutic possibilities by indirectly enhancing cellular protein-folding capacity (46).

The clinical approval of migalastat for Fabry disease provided the first proof-of-concept for pharmaceutical chaperone therapy in humans and demonstrated its potential as a disease-modifying treatment (47). Since then, several pharmaceutical chaperones have entered clinical development for

lysosomal storage disorders, cystic fibrosis, Parkinson's disease, Alzheimer's disease, and other protein misfolding disorders (48). Consequently, pharmaceutical chaperones are now recognized as a promising therapeutic strategy for the treatment of both rare and common conformational diseases (49).

4. Mechanism of Action of Pharmaceutical Chaperones

The therapeutic effectiveness of pharmaceutical chaperones is based on their ability to stabilize misfolded or conformationally unstable proteins and restore their normal biological function. Many disease-causing mutations do not completely eliminate protein activity but instead impair protein folding, stability, and intracellular trafficking. Pharmaceutical chaperones bind selectively to these proteins, promoting proper folding and functional expression (50).

4.1 Protein Stabilization and Folding

Pharmaceutical chaperones stabilize partially folded proteins by binding to active sites, substrate-binding regions, or allosteric sites, thereby enhancing structural integrity and reducing susceptibility to degradation (51,52). During protein maturation in the endoplasmic reticulum, these molecules assist folding intermediates in achieving their native conformation and decrease the likelihood of aggregation and misfolding (53,54).

4.2 Prevention of Degradation and Restoration of Trafficking

Misfolded proteins are commonly targeted by the endoplasmic reticulum-associated degradation (ERAD) pathway for proteasomal destruction. Pharmaceutical chaperones help mutant proteins escape ERAD by improving their structural stability and enabling them to pass cellular quality-control checkpoints (55,56). Consequently, these proteins can be transported through the Golgi apparatus to their functional destinations, such as lysosomes or the plasma membrane, where normal biological activity is restored (57,58).

4.3 Active-Site and Allosteric Mechanisms

Many first-generation pharmaceutical chaperones function as active-site ligands that reversibly bind target enzymes and stabilize them during intracellular transport. Migalastat, used in Fabry disease, is a well-known example of this mechanism (59,60). Alternatively, allosteric chaperones bind regions distinct from catalytic sites and stabilize protein structure without directly interfering with enzymatic activity, offering improved selectivity and broader therapeutic applicability (61,62).

4.4 Proteostasis Regulation and Therapeutic Impact

Some pharmaceutical chaperones act indirectly by modulating proteostasis networks, including molecular chaperones, stress-response pathways, autophagy, and degradation systems. These proteostasis regulators improve the cellular environment for protein folding and reduce protein aggregation (63,64). By decreasing the formation of toxic aggregates and restoring functional protein levels, pharmaceutical

chaperones can alleviate cellular stress and improve disease outcomes, particularly in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (65,66).

Overall, pharmaceutical chaperones restore endogenous protein function through stabilization, correction of folding defects, prevention of premature degradation, and recovery of intracellular trafficking. These mechanisms directly address the molecular basis of conformational diseases and represent a promising precision-medicine approach for the treatment of both rare genetic and common neurodegenerative disorders (67,68).

5. Classification of Pharmaceutical Chaperones

Pharmaceutical chaperones are classified according to their mechanism of action and interaction with target proteins. This classification is important for understanding their therapeutic applications in protein misfolding disorders (69).

5.1 Active-Site Specific Chaperones

These chaperones bind reversibly to the catalytic site of target proteins and stabilize their native conformation during folding and intracellular trafficking. They are widely used in lysosomal storage disorders, with migalastat being a notable example for Fabry disease (70).

5.2 Allosteric Chaperones

Allosteric chaperones bind to sites distinct from the active site and promote conformational stabilization without directly inhibiting protein function. This approach offers improved selectivity and reduced competition with endogenous substrates (71).

5.3 Chemical Chaperones

Chemical chaperones are non-specific small molecules that enhance protein stability and cellular folding capacity. Common examples include glycerol, dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO), and 4-phenylbutyrate (72).

5.4 Proteostasis Regulators

Proteostasis regulators indirectly improve protein folding by modulating cellular pathways involved in protein synthesis, trafficking, autophagy, and degradation. These agents enhance the overall protein quality-control network and support the stabilization of multiple proteins simultaneously (73).

5.5 Chaperones for Membrane Proteins

Certain pharmaceutical chaperones specifically target membrane receptors, ion channels, and transport proteins, facilitating their proper folding and trafficking. Such approaches have shown promise in disorders such as cystic fibrosis and receptor-associated diseases (74).

Overall, pharmaceutical chaperones may act through direct stabilization of target proteins or indirect modulation of cellular proteostasis pathways, providing diverse therapeutic options for conformational disorders.

6. Therapeutic Applications of Pharmaceutical Chaperones

Pharmaceutical chaperones have demonstrated considerable therapeutic potential in diseases associated with protein misfolding, instability, and defective intracellular trafficking. By restoring endogenous protein function, these agents offer a targeted approach for the treatment of several genetic and neurodegenerative disorders (75).

6.1 Lysosomal Storage Disorders

Among the most successful applications of pharmaceutical chaperones is the treatment of lysosomal storage disorders. In Fabry disease, migalastat stabilizes mutant α -galactosidase A, enhances lysosomal trafficking, and restores enzymatic activity (76). Similarly, in Gaucher disease, compounds such as ambroxol improve glucocerebrosidase stability and reduce substrate accumulation (77). Pharmacological chaperones have also shown promise in Pompe disease by enhancing the stability and effectiveness of acid α -glucosidase and enzyme replacement therapies (78).

6.2 Cystic Fibrosis and Neurodegenerative Disorders

In cystic fibrosis, pharmacological correctors improve the folding and trafficking of mutant CFTR proteins, thereby restoring chloride channel function (79). In neurodegenerative disorders, pharmaceutical chaperones have attracted attention due to their ability to reduce protein aggregation and improve cellular homeostasis. Ambroxol has shown potential in Parkinson's disease through enhancement of glucocerebrosidase activity and reduction of α -synuclein accumulation (80). Likewise, chaperone-based approaches are being investigated for Alzheimer's disease to limit β -amyloid and tau aggregation (81).

6.3 Other Emerging Applications

Beyond genetic and neurodegenerative diseases, pharmaceutical chaperones are being explored for the stabilization of tumor suppressor proteins and correction of enzyme deficiencies in metabolic disorders. Their expanding range of targets highlights their potential in precision medicine and personalized therapeutics (82).

7. Advantages and Limitations of Pharmaceutical Chaperones

Pharmaceutical chaperones offer several advantages over conventional therapies because they directly address the molecular defects responsible for protein misfolding disorders (83).

7.1 Advantages

These agents restore endogenous protein function rather than replacing proteins externally, thereby preserving normal cellular regulation (84). Most pharmaceutical chaperones are orally active small molecules with good tissue penetration and intracellular accessibility (85). They also exhibit lower immunogenicity than biologic therapies and can be tailored to specific mutations, making them valuable tools in personalized medicine (86,87).

7.2 Limitations

Despite their promise, pharmaceutical chaperones are often mutation-specific and therefore effective only in selected patient populations (88). Active-site chaperones may occasionally interfere with enzyme activity, and long-term safety data remain limited for many investigational compounds (89,90). Furthermore, their effectiveness may be restricted in diseases involving severe protein loss or irreversible tissue damage (91).

8. Conclusion

Protein misfolding is a major factor in the development of numerous genetic and acquired diseases. Pharmaceutical chaperones have emerged as a promising therapeutic approach by stabilizing misfolded proteins, restoring their proper folding and trafficking, and improving functional activity.

The clinical success of migalastat in Fabry disease has demonstrated the potential of pharmaceutical chaperone therapy and encouraged its investigation in other disorders, including neurodegenerative, metabolic, and lysosomal storage diseases. Advances in structural biology, artificial intelligence, and precision medicine are expected to accelerate the discovery of more effective chaperone-based therapies.

Although challenges such as mutation specificity and long-term safety remain, pharmaceutical chaperones represent an important class of targeted therapeutics with significant potential for the future treatment of conformational diseases.

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Pharmacological evaluation of carbazole derivatives

Carbazole derivatives are there different type of biological activities.[1] It is evident from the many type of experiment that the derivatives of carbazole possess a wide spectrum of biological activities, such as antitumor, antibacterial, antifungal, antineoplastic, anticonvulsant, antioxidant, anti-diabetic, antipsychotic, Anti-emetic, larvicidal activity and Anti HIV etc.[2] Various heterocyclic carbazole derivatives present because of some their natural occurrence and the different type of broad spectrum of pharmlological activity releted with these compounds. The carbazole component is a frequent moiety of various drugs such as carvedilol, carprofen,

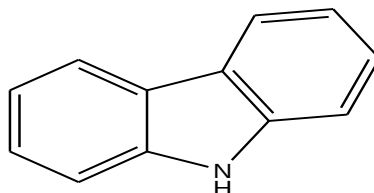
carazolol, flucindole, rimcazone, ciclindole, nmp-7, rimcazone, latrepirdine, olivacine, ellipticine, celliptium etc are introduced. Statins are also known as HMG-CoA re-educates inhibitor and lipid lowering drugs. Still the beneficial effects of statins in forbidding cardiovascular diseases may evolve from their lipid-lowering activity.[3] In recent year carbazole and its derivatives have been extensively investigated and have become one of highly moiety are produced and progress are quite rapid. In case of anti-bacterial, antifungal and antitumor etc have been quickly studied and show great potential and pharmacological effect. [4]

Carbazole

Carbazole is one of aromatic organic hetero cyclic compounds. Carbazole has a tricyclic structure consisting of two six- member benzene rings on either side of the rings five member nitrogen. Carbazole is also a component of tobacco smoke.

Its, other name is dibenzopyrrole, diphenyleinimine, 9-azafluorene, and IUPAC name 9H-Carbazole and Chemical formula $C_{12}H_9N$. [5]

The prevasiveness of heterocyclic compounds in medicinally important, continues to derive the need for new methods for their preparation1. [6] Carbazole and its derivatives are known to have important biological properties. First of all to carbazole was isolated from coal tar.



Carbazole

In this review, the authors summarize different synthetic routes for the syntheses of Carbazole and their derivatives. Moreover, a detailed coverage from year 2013-March 2020 of the classical methods and the non-classical procedures for the all syntheses of pharmacological active Carbazole and their derivatives have also been reported. [7] The nomenclature used in these Carbazole derivatives is reviewed by chemical abstract. The carbazole term used in this review mean 9H- Carbazole.

Chemistry of Carbazole

Carbazole is a polynuclear hydrocarbon organic compound. Carbazole has a tricyclic structure consisting of two six- member benzene rings on either side of the rings five member nitrogen.

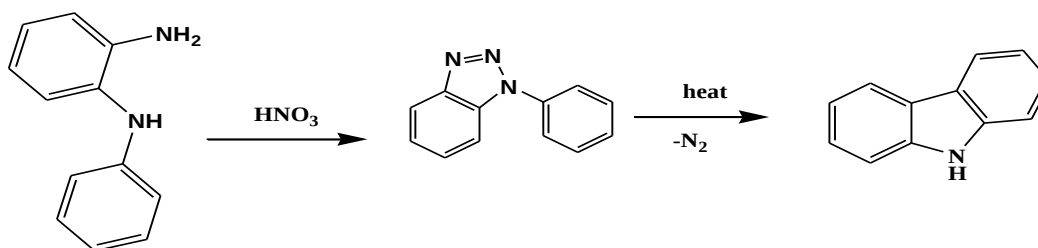
The structure of this compound is based on the structure of indole in which the second benzene ring is joined with a five-member ring at the 2, 3 position of the indole. (Similar to a 4, 9 double bond position in carbazole). [9]

Carbazole is aromatic tricyclic structure it is mostly build from coal tar. Carbazole is a raw product for the top class strain of violet 23 and it is widely used in repair varnishes, car varnishes, printing paper inks, polymers and textiles industry. Carbazole derivatives have gained the observation of researchers due to their pharmacological activity against different types, like cell proliferation, neurological disorders, anti-inflammation etc.[10] In the reference of medicinal importance carbazole are latterly synthesized a different types of derivatives.

Preparation of Carbazole

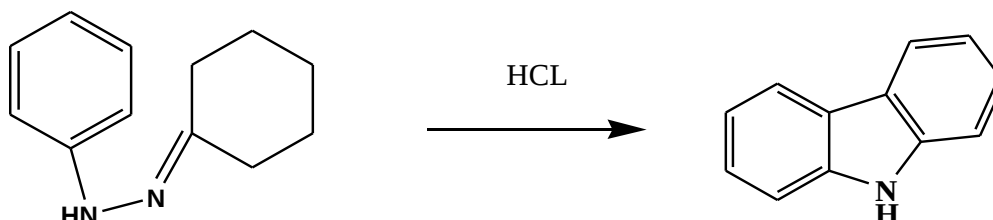
Bucherer carbazole synthesis:

Firstly 2-aminodiphenylamine is converted into a diazonium salt which immediately forms a 1, 2, 3-triazole. This triazole is unstable form and at high temperatures, nitrogen is set in a free form and produced carbazole. [11]



Borsche–Drechselcyclization:

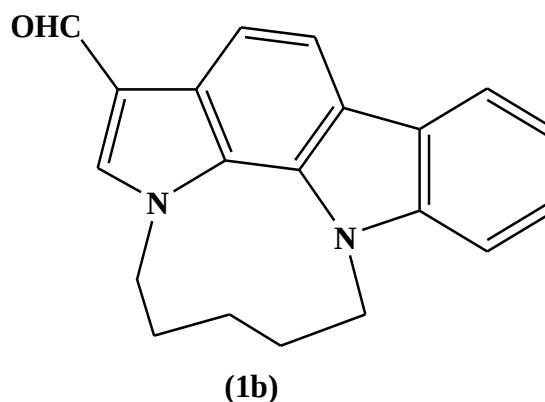
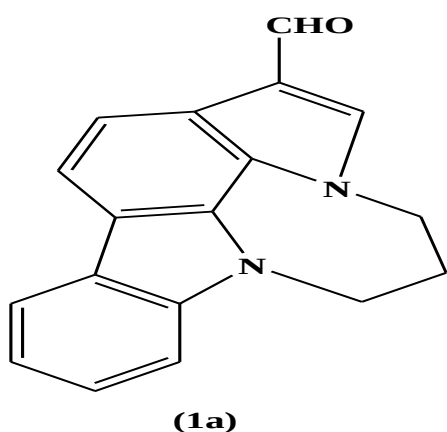
Synthesis of Carbazole from the process of chemical and biological cyclization of phenylhydrazine is concised with cyclohexanone to the identical imine in the laboratory. After that second step ring closing reaction take place with hydrochloric acid and produced tetrahydrozole. These two steps are modification step, involve into one by one in third step, compound of acidic acid is oxidized itself to carbazole.[12]



Literature surveys have proclaimed various therapeutic uses of carbazole and its derivative. The major pharmacological classes under which carbazole derivative can be classified.

1. Leukemiatherapy:

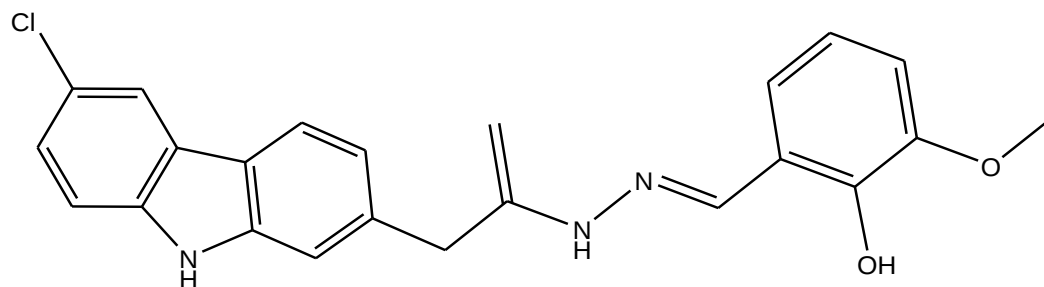
Giraud et al. 2015 N1-N10-bridged pyrrolo carbazoles have synthesized. The ability of this product has ability to inhibit serine/threonine kinase. Compound 1a and 1b show potent activity against the acute myeloid leukemia ipc-81 with inhibitory potency, which is a good predictor of leukemia therapy.



et al. 2023 optimize carbazole derivatives as potential cytotoxic agents. All synthesized carbazole derivatives were evaluated for their in vitro cytotoxic activity against gastric adenocarcinoma, human melanoma and African green monkey kidney cell lines.

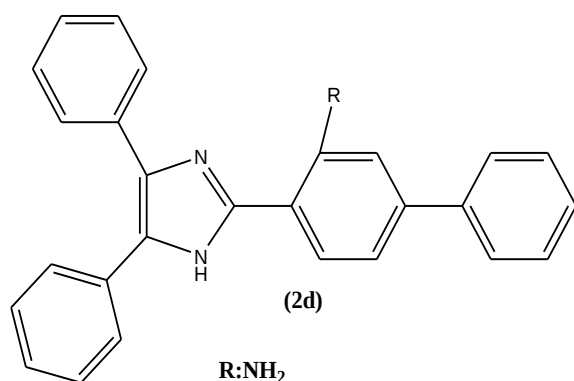
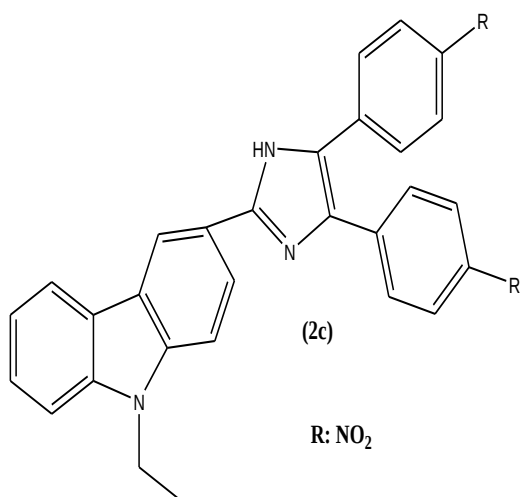
The preliminary results indicated that compound 2a exhibited high inhibitory activities against gastric adenocarcinoma, human melanoma and African green monkey kidney cell lines.

Gao et al. and their colleagues discovered potent lead moiety and checked their instrumental data for chemical evaluation and checked pharmacological response for cancer cell.



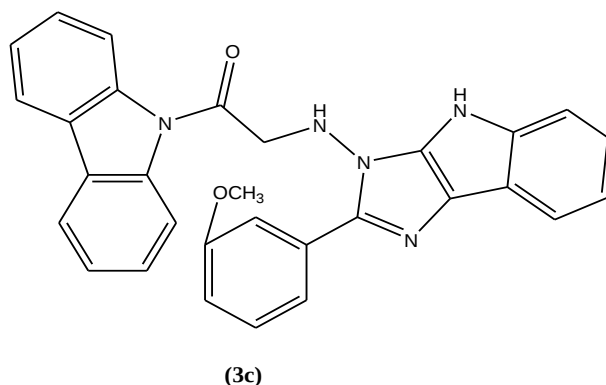
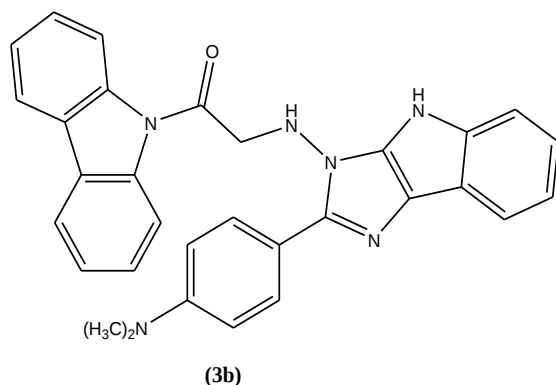
(2a)

Behbood Taheri et al., Mehdi Taghavi et al. 2020 synthesized the different carbazole derivatives and evaluated their cytotoxicity against 60 human tumor cell lines. Heterocyclic compound 2c & 2d show good anticancer activity among the compounds. Cancer is the most dangerous and mortal diseases in the world. Carbazole containing compound NO₂ group had higher anticancer effect than NH₂ group against all tested cell lines.



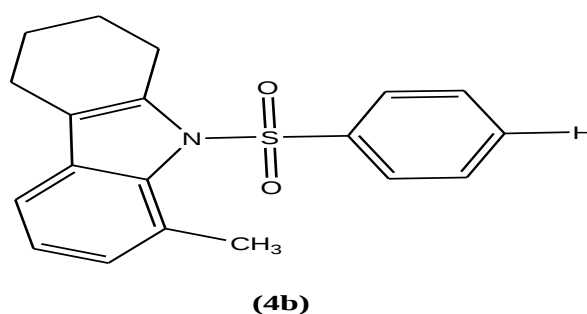
2. Antibacterial and Antitumor activity

Kumar N *et al.* 2013 For antibacterial and antitumor activity two gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* were taken, firstly a carbazole derivative and synthesized by in vitro studies. [14] Synthesized carbazole moiety show good antibacterial activity against two gram negative bacteria. Activity of those compounds 3b and 3c were most potent against positive and negative bacterial strains. Those compound show electron donating groups therefore bacterial activity was increase. For Anticancer activity, compound 3b & 3c were synthesized and evaluated by determining the percentage growth inhibition of Ehrlich's. And found good activity against tumor cell. These compounds have electron donating group which increase the antitumor activity of compound. [15]



3. Antioxidant activity

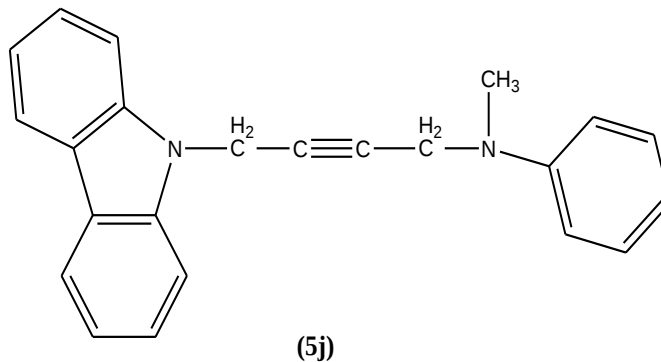
SubrahmanyamL *et al*2016 have synthesized A New Class of N-Substituted Tetrahydrocabazole derivatives by in presence of reagents like glacial acetic acid leads the formation of Intermediate substituted compounds tetrahydrocabazole, cyclohexanone and phenyl hydrazine's using as starting material. When treated with 10% sodium hydroxide the intermediate compounds and substituted 4 amino-benzoyl chloride give 1, 2, 3, 4 tetra hydrocarbon derivatives (4a to 4d). The structures of the new derivatives were characterized by NMR, IR and mass spectral data. Among all synthesized newly compounds were evaluated in vitro study show 4b compound good anti-oxidant activity due to methyl group present at position 8th of this compound.



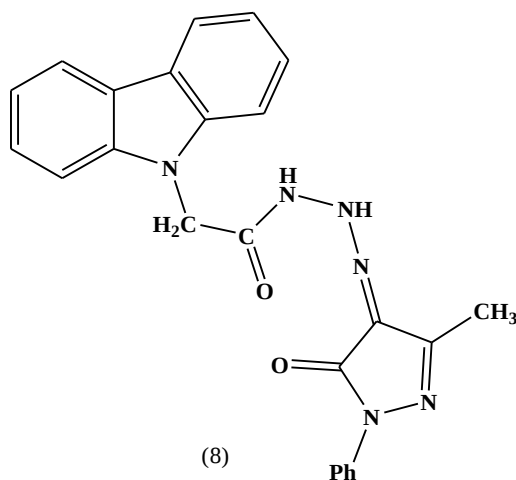
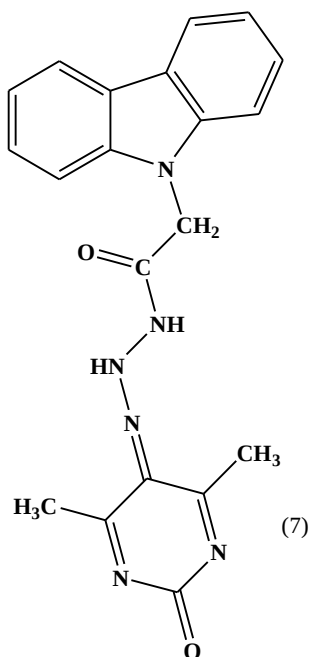
4. Antimicrobial Activity

Al.Kaissy W.N Wafaa *etal*.2013 synthesized newly compound of antimicrobial activity was listed. The all prepared compounds [5a to 5j] possess moderate activity against all types of bacteria and candidau while the compounds [5a, 5b, 5b, 5c and 5h] possess weak activity against bacteria strain. As far as compound [5j] possess very high specific activity against (E. coli).

Bacteria and one yeast. Some of the prepared compounds for such bacteria and yeast in study have moderate to high activity. [17]

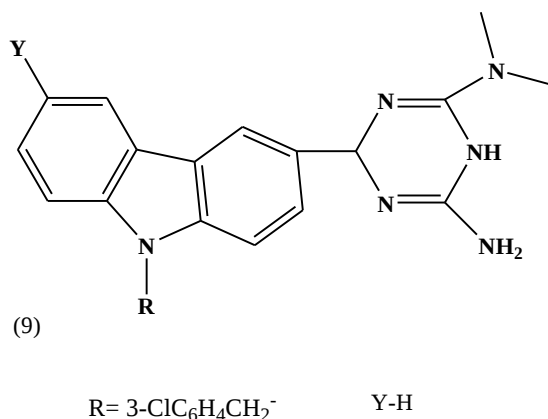


Sahil nadia, Salimon jumat et al. 2011 synthesized different carbazole derivatives. All synthesized compounds were characterized by NMR, IR, elemental analysis, All the newly synthesized compounds were evaluated for their in vitro antibacterial activity against gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). Compounds 7, 8 shown good antimicrobial activities.



Xue yi-jie, Li ming- yue *et al.* 2020 design and synthesized five series of carbazole derivatives isonicotinic, semicarbazide, thiosemicarbazide, dihydrotriazine & amino guanidine.

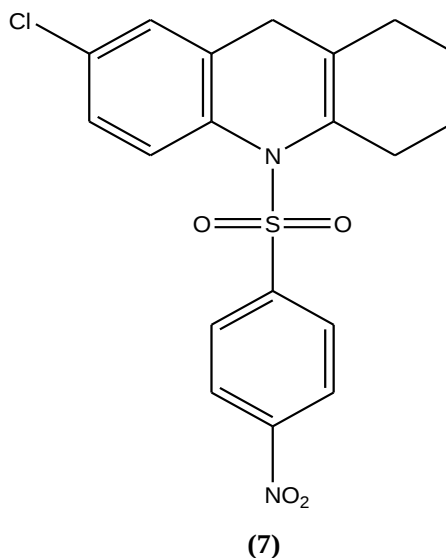
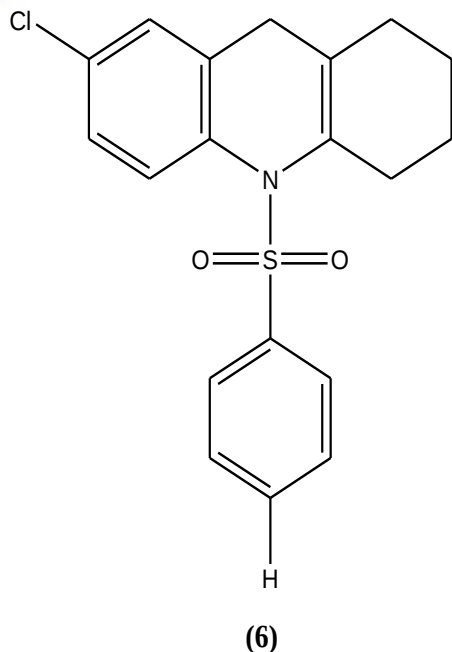
The synthesized compounds 9 inhibit the different bacterial microorganism strain and fungal strain due to increases their lipophilic character of molecules.



5. Anti-bacterial activity

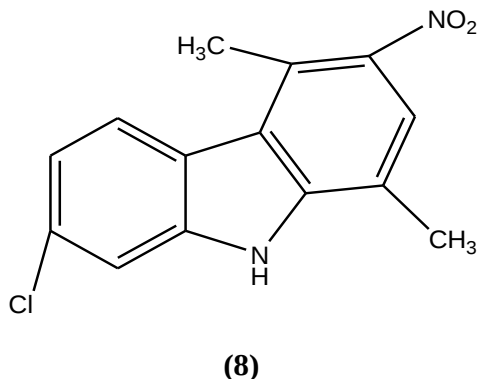
Sakinala Padmavathi *etal.*2016, the nomenclature of heterogeneous organic compounds uses a compound called tetrahydrocarbazole. The preparation of substituted tetrahydrocarbazole derivatives from cyclohexanone & phenyl hydrangean in glacial acetic acid using reflux condenser. These intermediates were definitely converted to N-substrate tetrahydrocarbazole upon reaction with the aromatic acid chloride substituted in the alkaline medium.

1 to 15 compounds are synthesized and check there characterized by IR, NMR, MS, MP and elemental analysis. All those compounds having docking studies and activity was checked by agar cup plate method. Compound 6&7 having shown good anti-microbial response against bacterial strain. These studies give better response for further synthesis of carbazole derivatives [18].



6. Anti HIV

Saturnino Carmela, Grande Fedora et al. 2018, AIDS is a dangerous and most serious infection causes by human immunodeficiency virus (HIV), it may causes death. Human immune deficiency virus Causes acquired immune deficiency syndrome (AIDS).[19] Many carbazole derivative are reported potent anti-HIV agent which inhibit the integrase enzyme. There are different types of derivatives achieved by selecting well in-vitro study and antiviral study and reduce their side effects. Many carbazole derivatives are inhibiting the growth of HIV and sexual transmitted deases, now a day discovered new entity to treat the chronic lifelong infection. Six chloro-1,4-dimethyl-9H-carbazoles have prepared and modified by using chemical and tested in luciferase and *Escherichia coli* β -galactosidase expressing CD4⁺, CXCR4⁺, CCR5⁺ T2M-bl cells. A primary biological investigation on the synthesized small series of chloro-1,4-dimethyl-9H-carbazoles has been carried out. Among all the compounds and was passing all test procedure, nitro derivative compound (8) having good representing the anti-HIV response against virus strain. These studies give better response for further lead the developed new anti-HIV agents.



7. Rheumatoid arthritis

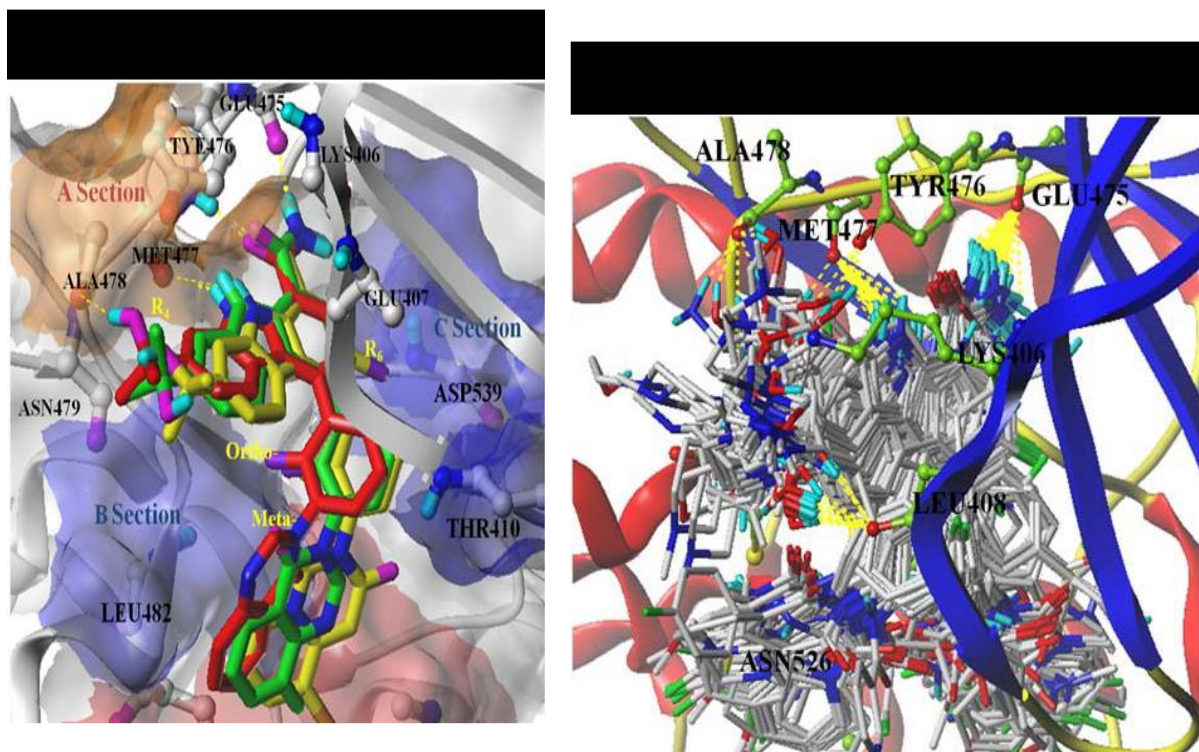
Rheumatoid arthritis is a type of arthritis where weak your immunity power like joint including in hands and feet. Rheumatoid arthritis mostly affect the internal organ of joining side and causes pain with full swelling.[20] *Bankim ChandraNandy et al* 2018 was reported Carbazole derivatives have anti-inflammatory activity and it was work on the specific side receptor antagonist know serotonin (5HT3). Carbazole derivative are used as non-steroidal anti-inflammatory drug (NSAID) and other veterinarians as a supportive treatment for the relief of arthritic symptoms.

Rheumatoid arthritis (RA) is the second common arthritis disease with chronic, rheumatic inflammatory features. [21] Non-steroidal anti-inflammatory drugs, slow-acting anti-rheumatic drugs and glucocorticoid drugs can improve the symptoms of rheumatoid patients, but fail to treat. Breton's tyrosine kinase (BTK) inhibitors have been shown to be an effective target against autoimmune signals and B-cell malignancies.

. Among the current clinical drugs, only Bristol- Myer Squibb (BMS-986142), classified as a carbazole derivative, is used for treating RA. Design to novel and highly potent carbazole inhibitors, molecular docking and three dimensional quantitative structure–activity relationship (3D-QSAR) were applied to explore a dataset of 132 new carbazole carboxamide derivatives. The established comparative molecular field analysis (CoMFA) ($q^2 = 0.761$, $r^2 = 0.933$) and comparative molecular similarity indices analysis (CoMSIA) ($q^2 = 0.891$, $r^2 = 0.988$) models obtained high predictive and satisfactory values. CoMFA/CoMSIA contour maps demonstrated that bulky substitutions and hydrogen-bond donors were preferred at R1 and 1-position, respectively, and introducing hydrophilic substitutions at R1 and R4 was important for improving BTK inhibitory activities. These results will determine to the design of novel and highly potent BTK inhibitors.

The accuracy of the docking program was confirmed by comparing the predicomparing the predicted compound (green) and ligand (red) extracted from the crystal structure of BTK

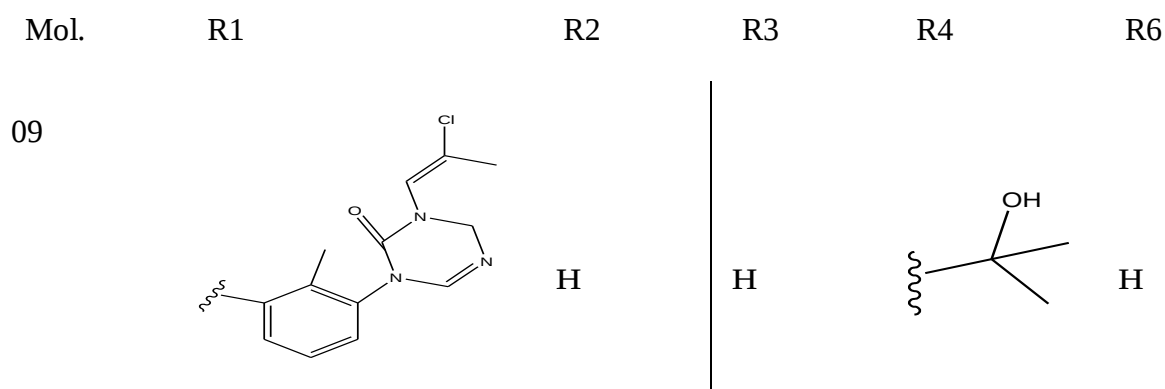
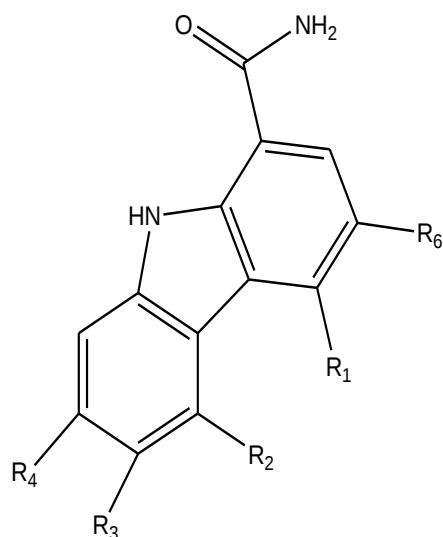
(Protein Data Bank ID: 5JRS). The result, revealing excellent agreement, is shown in Figure and confirms that the selected experimental parameters and procedures used for molecular docking and alignment were reasonable. As depicted in Figure, the common carbazole rings of **9** and **10** as well as experimental ligand were in the same position and mainly interacted with residues.



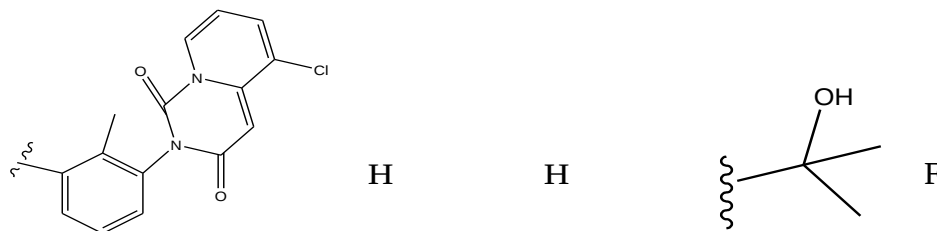
An X in the figure; Binding posture prediction of **9** (green) compared to ligand (red) found in ray crystal structure; **10** (yellow) position in the active place of the protein and the binding pocket of the BTK enzyme. (B) of dataset molecules. Sequence based. Hydrogen bonds are represented as yellow lines, and the main protein residues are labeled with ball and stick forms. Section A: Hinge area, sections B and C; Hydrophobic Pocket, Section D; Floor loop.

To explain the binding mode, **9** (IC₅₀ = 0.22 nM) was selected for more detailed analysis, since it was the most representative inhibitor in the active site of the protein. Based on Figure, the carbazole ring of **9** interacted with =C=O and N–H of Met 477 and =C=O of Gly 475 by hydrogen bonds in the hinge region, and interacted with the benzene ring of Tyr 466 by a conjugate effect; among them, Gly475 and Met477 are two significant gatekeeper residues in BTK enzyme. The hydroxyl group at R4 also had a hydrogen-bond interaction with Ala478. Chlorine atom at R6 formed a hydrophobic interaction with Glu407 and Asp539. The benzene ring's ortho-groups at R1 also interacted with Cys527 and Leu528 through a hydrophobic effect. At the bottom, the

substituent of meta-position of the benzene ring was well filled in a floor loop formed by Asn484, Leu483, and Arg525. All these action characteristics proved that **9** was the most active molecule in the dataset.



10

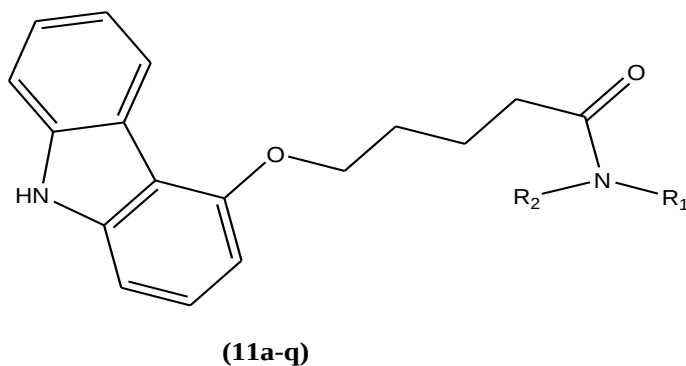


8. Anti-inflammatory & antioxidant activities

Govindhan Muniyappan *et al* 2016 Synthesis of carbazole derivatives, it is good anti-inflammatory & antioxidant activity compounds 11a–q has been reported in good activity. The reaction proceed by O-alkylation of 4-hydroxycarbazole results in which on hydrolysis succeed by coupling reaction with different primary and secondary amines geted to the formation of new products (11a–q) in presence of methyl 5-bromovalerate. All those synthesized compounds 11a–q was character determine by using NMR, IR and mass spectral analysis. According to in-vitro study all synthesized compound have good anti-inflammatory and anti-oxidant activity using phosphomolybdenum & albumin denaturation methods, step by step. [22]

Standard drug Diclofenac sodium use as a reference. The new synthesized anti-inflammatory drugs (4h&4j) shown potent activity denoted by Fig.

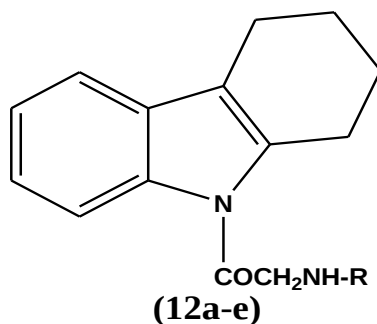
The compounds 11a–q was determined by ascorbic acid as the standard, the obtained results are shown in figure using phosphomolybdenum method. Anti-oxidant activities of new compounds compared with standard drug, ascorbic acid. Among the newly synthesized compounds 11m, 11p and 11q shown good anti-oxidant activity.



Mol.	R1	R2
11h	Hydrogen	Thiophene-2-ethyl
11j	Hydrogen	Prop-2-yl
11m	(4-Methanol)-1-piperidinyl	NA
11p	1-(4-N-methylpiperazinyl)	NA
11q	1-(Morpholinyl)	NA

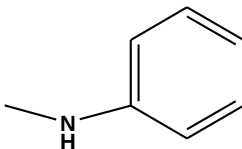
9. Antifungal activity

Devendar pathak et al 2014, A number of novel carbazole derivatives with starting material of carbazole, produced ethyl 2-(9H-carbazole-9-yl)acetate (1) which on the treated with ethyl chloroacetate. This compound in presence of sulphuric acid react with semicarbazide and produced 5-((9H-carbazole-9-yl)-1,3,4-oxadiazol-2-amine, which on reaction with piperazine and a different aromatic aldehydes in the presence of acetic acid yielded the yield different compounds (12a-o).[23] The characteristic of compounds were determine by ¹H-NMR, IR, and mass spectral studies, by elemental analysis and Melting point. Reaction of material checked by the TLC profile. According to in-vitro study synthesized compounds 12a, 12d, 12e have good among the tested compounds have significant antifungal activity.

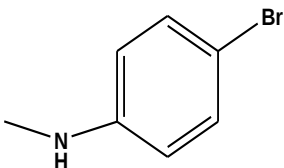


R

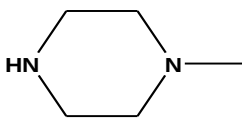
4a



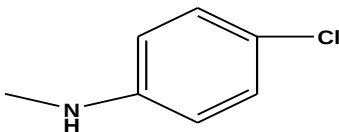
4b



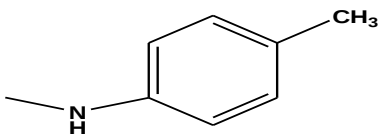
4c



4d

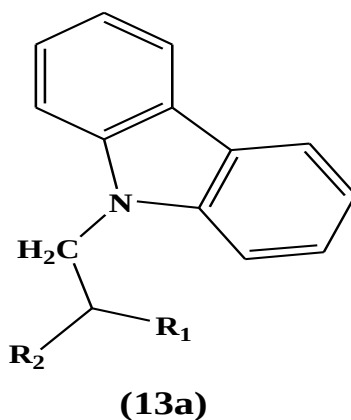


4e

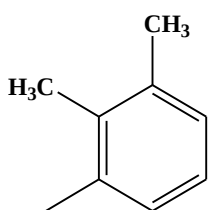


10. Anti-epileptic and antinociceptive activities

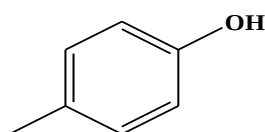
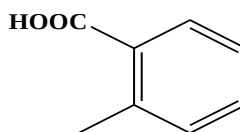
First time around in 1910 studies of phenobarbital showed good antiseizure activity used to induced sleep, it works many year but after time to time many drugs showing resistance in against of epileptic patients. [24] Time to time researchers was trying to synthesize more active and very less toxic drugs to control the seizures in human being and manufacture produced more drugs to control seizures to easy and more snug life for the patients. *Rjamanickam et al. 2015* introduced N-substituted carbazole derivatives and were in-vitro study of compounds, and screening for their antinociceptive & antilepileptic activities. Compound 13a showed great considerable anti-epileptic activity. This is due to the scompound, 2-(2,-3-dimethyl-phenyl)-amino-benzoic acid. The compound 13b have been passes antinociceptive & antilepileptic properties, reported compound details were given in Table.

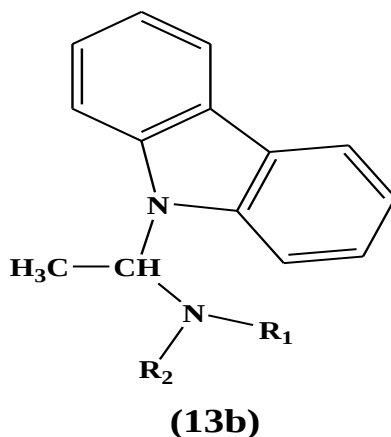


R1

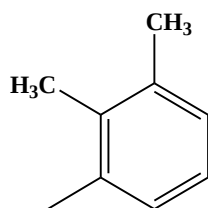


R2

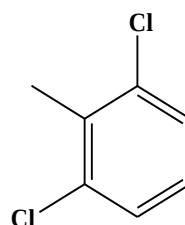
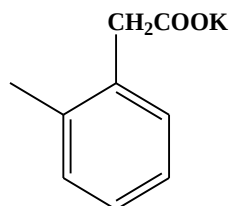
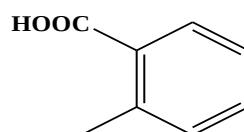




R1



R2

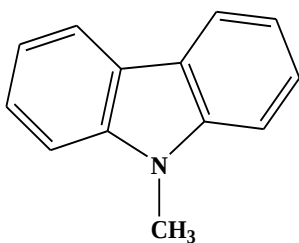


11. Anti- diabetic activity

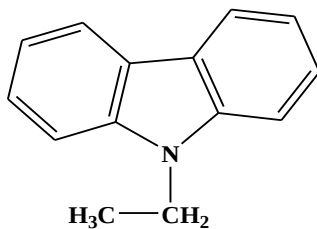
Eseyin A Olorufemi et al. 2017, Diabetes mellitus (DM) is a metabolic disorder in which person have high blood sugar, in this pancreas does not produced enough insulin. It is Dangers for human being cause of death. Diabetes mellitus produces classical symptoms like polydipsia, polyuria and polyphagia. In developed countries due to diabetic leading causes of heart attack, blindness, kidney failure and lower limb disorder. According to International Diabetes confederacy that about 331 million people will be diabetic by the year 2026. Mostly uses therapeutic approach for hyperglycemia by decreasing the rise in blood glucose concentration. This is achieved by maintaining and reducing the digestion and absorption of carbohydrate consumption through inhibition of carbohydrate hydrolyzing enzymes such as α -amylase and α -

glucosidase. Thus, the discovery of new clues for antidiabetic drugs with lower cost, better efficiency and less toxic has therefore become a matter of great priority.

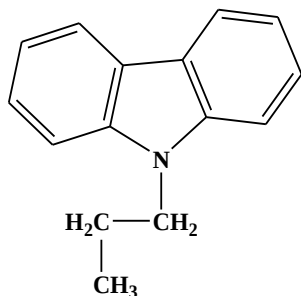
25] Different types of carbazole derivatives like methylcarbazole, ethylcarbazole, propylcarbazole and butylcarbazole were synthesized by alkylation of carbazole. In vitro study of synthesized compounds on inhibiting the alpha amylase activity therefore reducing hyperglycemia. All synthesized compounds (14-17) show good and potent activity. Increasing chain length of alkyl group in carbazole, its activity are increases it means inhibitory effect is directly proportion to the chain length of the alkyl group.



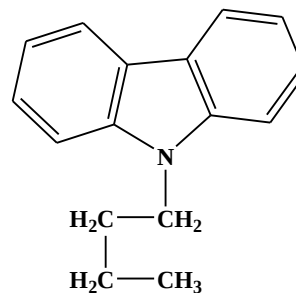
Methyl carbazole
(14)



Ethyl carbazole
(15)



Propyl carbazole
(16)

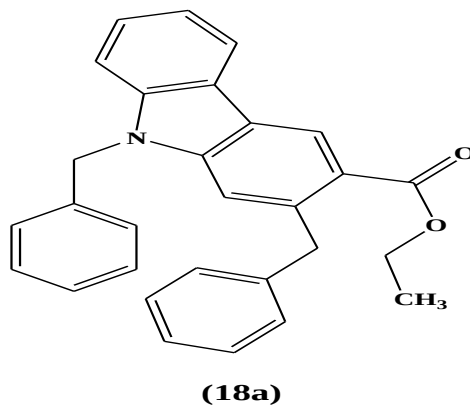


Butyl carbazole
(17)

12. Anti-proliferative effects:

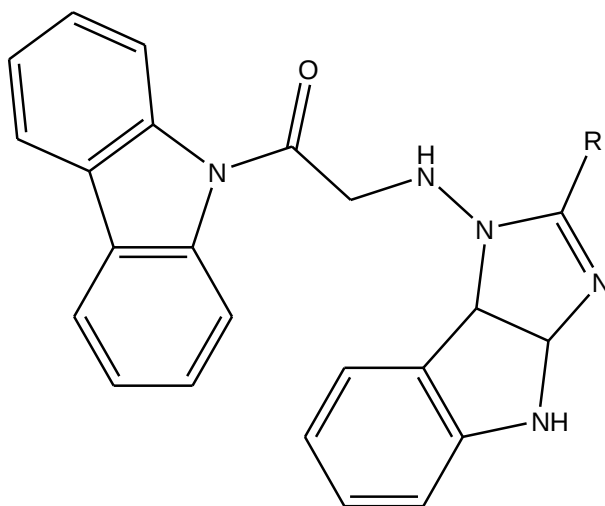
Chen et al. 2019, worked on the cancer cell (BC3EE2), the compound 18a (bis (carbazole-2, 9N-benzyl)-3-ethyl ethanoate) was synthesize. The synthetic carbazole derivative, upregulates autophagy and synergistically sensitizes human glioblastoma cells to temozolomide. In this study, anti-glioblastoma profiles of a series of synthetic carbazole derivatives were evaluated

invitro study. The most promising derivative in this series was BC3EE2, which showed significant anti-proliferative effects in cancer cells. [26]



13. Antibacterial activity and Antifungal activity

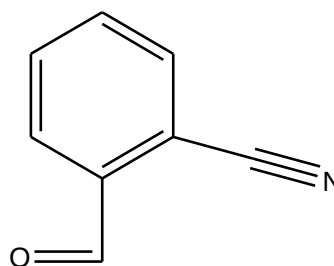
Reshma Hegden P et al. 2021 synthesized different types of novel N-(hydrazinoacetyl)-carbazole derivatives by using different types of software like ACD Lab and Chems sketch pharmacological activity was observed by the different software like admetSAR, molinspiration and PASS, Compounds 19 (C2 & C5) exhibit significance activity against both gram positive (*Staphylococcus aureus*) & gram negative (*Escherichia coli*) bacteria and 19c2 & c5 show antifungal activity against *Aspergillus niger* and *Candida albicans*.



(19)

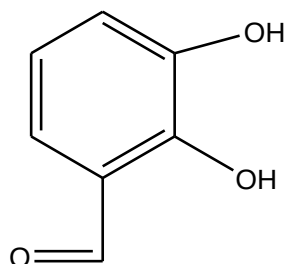
C2

R



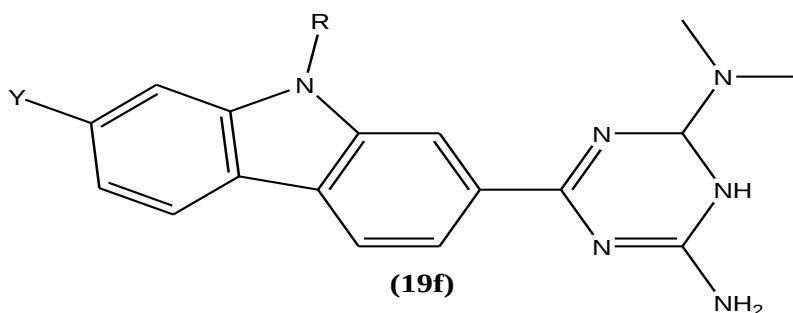
C5

R



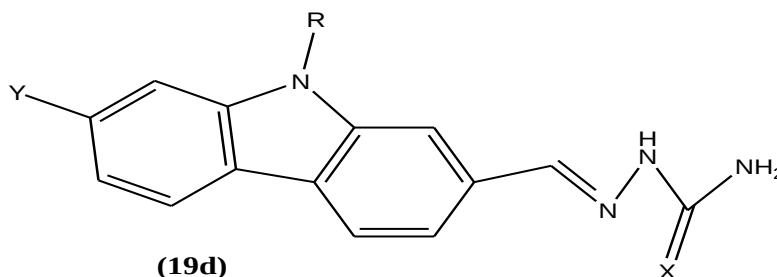
Yi-Jie Xue and Ming-Yue Li et al. 2021 synthesized carbazole derivatives with amino acid moiety and evaluated for activity against microbial agent. Compounds 19f & 19d showed the potent inhibitory Activities against Methicillin-resistant Staphylococcus aureus (Gram-positive bacterial) & Escherichia coli (Gram-negative bacteria) and antifungal activity against Candida

albicans during the investigation these compounds bound with block the active side of DHFR, in vitro study.



R: 2,4-Cl₂C₆H₃CH₂-

Y:H

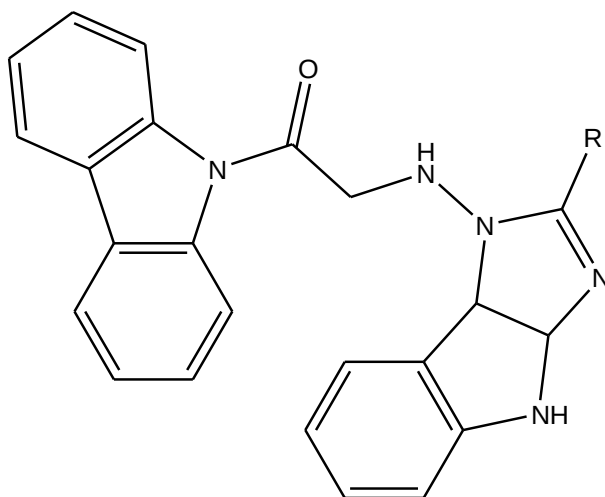


R: 4-CH₃C₆H₄CH₂-

Y:H

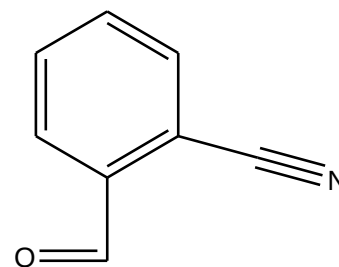
X:NH

Reshma Hegden P et al. 2021 synthesized different types of novel N-(hydrazinoacetyl)-carbazole derivatives by using different types of software like ACD Lab and Chemskech pharmacological activity was observed by the different software like admetSAR, molinspiration and PASS, Compounds 19g exhibit significance activity against both gram positive (*Staphylococcus aureus*) & gram negative (*Escherichia coli*) bacteria and 19h show antifungal activity against *Aspergillus niger* and *Candida albicans*.



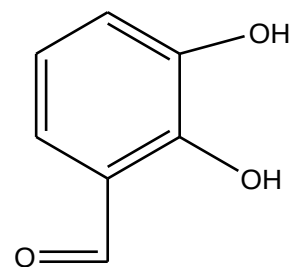
19g

R



19h

R



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