



A review on Agmatinase inhibitors

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Abstract : Agmatine is the product of arginine decarboxylation and can be hydrolyzed by agmatinase to putrescine, the precursor for biosynthesis of higher polyamines, spermidine, and spermine. Besides being an intermediate in polyamine metabolism, recent findings indicate that agmatine may play important regulatory roles in mammals. Agmatine, 4-aminobutyl guanidine, has recently been found in various mammalian organs and is thought to act as a neurotransmitter or neuromodulatory agent. The present study is to do a review on agmatine and its synthesized analogues till now for agmatinase inhibitory action. Agmatinase is a binuclear manganese metalloenzyme and belongs to the ureohydrolase superfamily that includes arginase, formiminoglutamase, and proclavaminase amidinohydrolase. Compared with a wealth of structural information available for arginases, no three dimensional structure of agmatinase has been reported. Agmatinase is an enzyme which blocks the mammalian agmatine which is ultimately responsible for the agmatine degradation in the body. Agmatinase is an enzyme which regulates the half life of agmatine in the brain. Hence a selective inhibitor of brain agmatinase is required. Several derivatives of agmatine are synthesized previously for agmatinase inhibitory activity but none of them showed selective inhibition. PZC (Piperazinecarboxamidine) is a derivative of agmatine or guanidine is expected to show selective inhibition of human agmatinase. A detailed review is carried out in order to understand the agmatinase inhibitor.

Key words - Agmatinase, Neurotransmitter, Neuromodulator, Guanidine, Piperazinecarboxamidine, Putrescine, arginase, Catecholamines, Nitric oxide synthase (NOS), N-methyl, D-aspartate (NMDA)

Introduction

Agmatine is an amine which is formed by the decarboxylation of L-arginine with the help of enzyme Arginine decarboxylase (ADC) and hydrolysed with the help of enzyme Agmatinase to putrescine [18]. It is a 4-aminobutyl guanidine, has recently been found in various mammalian organs and is thought to act as a neurotransmitter or neuromodulatory agent. It stimulates the release of catecholamines from adrenal chromaffin cells and increases the release of luteinizing hormone-releasing hormone from hypothalamus. The physiologic roles of agmatine in the central nervous system (CNS) are not well established. When Agmatine is exogenously administered to rodents, It enhances morphine analgesia, blocks tolerance to opioids, and attenuates withdrawal syndrome both in morphine and ethanol-dependent rats. It is a neuromodulator that is stored in Astrocytes and is transported to the Synaptosomes via. a selective, Na-independent uptake system. It binds to adrenoreceptors and imidazoline binding sites with high affinity. Agmatine blocks spinal nociceptive reflexes when administered systemically and reverses chronic pain

induced by inflammation, neuropathy, and spinal cord injury. Agmatine has its in neurodegenerative diseases and epilepsy. It is also released from neurons and has neuroprotective properties. In addition, agmatine can evoke a noncompetitive voltage- and concentration-dependent block of the N-methyl-D-aspartate (NMDA) ionophore and inhibits all isoforms of nitric oxide (NO) synthase. Both NMDA antagonists and NO synthase inhibitors are known to have anti-depressant-like effects in animal models 8, 11.

The agmatine has long been known to be a constituent of bacteria, plants, and a range of invertebrates. In mammalian brain, it is an endogenous ligand. Previous research indicates that this compound, which is not able to interact with opioid receptors, plays an important role in regulating the pharmacological actions of opioids. Agmatine has weak analgesic effects and shows biphasic modulation on opioid functions, which enhance opioid analgesia, but inhibit tolerance to and substance dependence on opioids. The mechanisms associated with the analgesic effect and biphasic modulation on opioid functions is by activation of imidazoline receptors on central nervous system. 10, 12.

Agmatine i.e. 4-aminobutyl guanidine. Guanidine is also called carbamidine, is a strongly alkaline and water-soluble compound that plays a key role in numerous biological activities. The guanidine group denotes chemical and physicochemical properties of many compounds of medical interest. Structural characterization of agmatine at physiological conditions Trimethoprim² 1, sulfadiazine³ 2, and Gleevec (imatinib mesilate) are examples of pharmaceutically important guanidine-containing heterocycles. In peptides, residue of arginine has a guanidine structure in the protonated form as guanidinium ion, which functions as ancient identification. moiety of anionic substrates such as carboxylate, nitronate, and phosphate functionalities. The guanidinium ion is also involved in many enzymatic transformations, because it can orient specific substrates based on their electronic characteristic and it is able to form a transition state assembly with the substrates to reduce the activation energy or to stabilize anionic intermediates 13. Guanidines form an important class of hetero atom containing organic compounds, found in natural products and constitute an important unit/building block in biological, agrochemical and pharmaceutically active compounds. 1 They act as ancillary ligands for stabilizing various metal complexes, including main group, transition and lanthanide metals and also as base catalysts 7.

Agmatine has properties that uniquely distinguish it from other monoamines because of the strong basic character of its guanidine group. Because of this property, it is unusual in being protonated under physiological conditions. Agmatine is present in low (pM–nM) concentrations in many organs, with enrichments in certain brain upon regions 6.

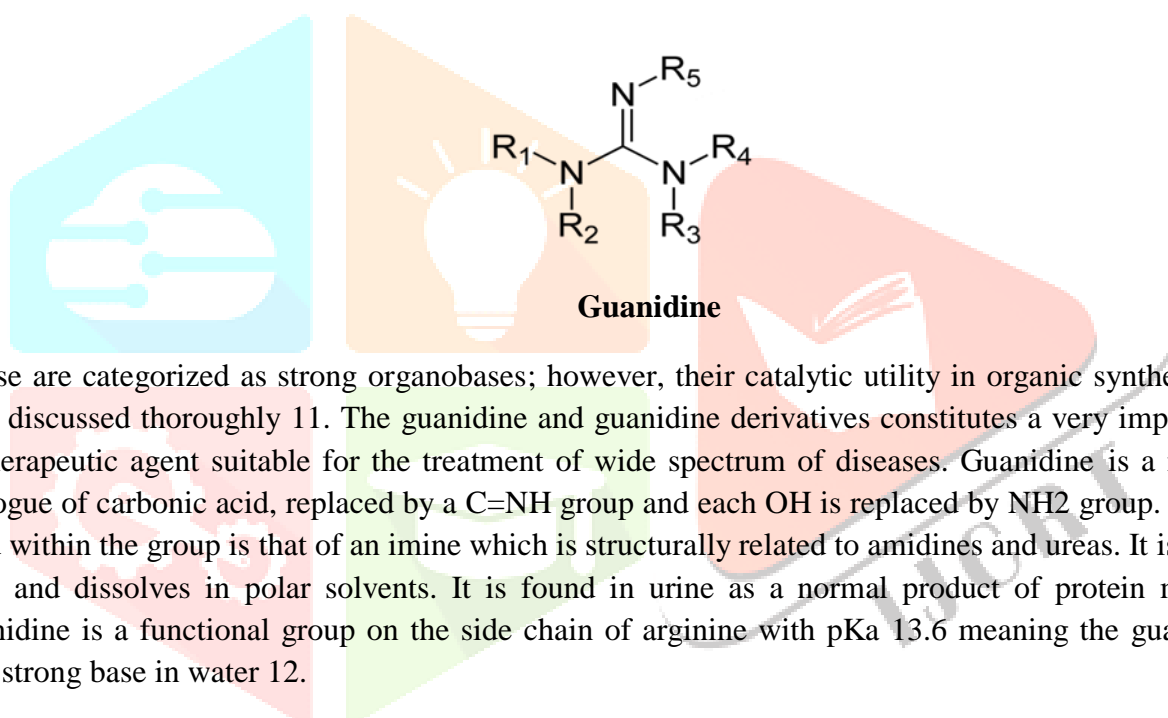
Agmatinase, the enzyme that hydrolyzes agmatine to form putrescine and urea in lower organisms, was found in rat brain 3. Agmatinase is a binuclear manganese metalloenzyme and belongs to the ureohydrolase superfamily that includes arginase, formiminoglutamase, and proclavamate amidinohydrolase. Compared with a wealth of structural information available for arginases, no three dimensional structure of agmatinase has been reported. Agmatinase from *Deinococcus radiodurans*, a 304-residue protein, shows 33% of sequence identity to human mitochondrial agmatinase. Here we report the crystal structure of *D. radiodurans* agmatinase in Mn²⁺-free, Mn²⁺-bound, and Mn²⁺-inhibitor-bound forms, representing the first structure of agmatinase. It reveals the conservation as well as variation in folding, oligomerization, and the active site of the ureohydrolase superfamily. *D. radiodurans* agmatinase exists as a compact homohexamer of 32 symmetry. Its binuclear manganese cluster is highly similar but not identical to the clusters of arginase and proclavamate amidinohydrolase. The structure of the inhibited complex reveals that inhibition by 1,6-diaminohexane arises from the displacement of the metal-bridging water 8.

Objective

- 1) To understand the physicochemical properties of agmatine and its role.
- 2) To know the importance of agmatine in mammalian body.
- 3) To find out different agmatine analogues which shows agmatinase inhibitor activity

Guanidine

Guanidine is the compound with the formula $\text{HNC}(\text{NH}_2)_2$. It is colourless solid and dissolves in polar solvents. It is strong base that is used in the production of plastic explosives. It is found in urine as a normal product of protein metabolism 9. Guanidines are a group of organic compounds sharing a common functional group with the general structure $(\text{R}_1\text{R}_2\text{N})(\text{R}_3\text{R}_4\text{N})\text{C}=\text{N}-\text{R}_5$. The central bond within this group is imine, and the group is related structurally to the amidines and ureas 10.



These are categorized as strong organobases; however, their catalytic utility in organic synthesis has not been discussed thoroughly 11. The guanidine and guanidine derivatives constitutes a very important class of therapeutic agent suitable for the treatment of wide spectrum of diseases. Guanidine is a nitrogenous analogue of carbonic acid, replaced by a $\text{C}=\text{NH}$ group and each OH is replaced by NH_2 group. The central bond within the group is that of an imine which is structurally related to amidines and ureas. It is colourless solid and dissolves in polar solvents. It is found in urine as a normal product of protein metabolism. Guanidine is a functional group on the side chain of arginine with pK_a 13.6 meaning the guanidine is a very strong base in water 12.

The highly basic nature and remarkable stability is conferred to the guanidinium skeleton on protonation by the so called Y aromaticity that is resonance through three canonical forms.

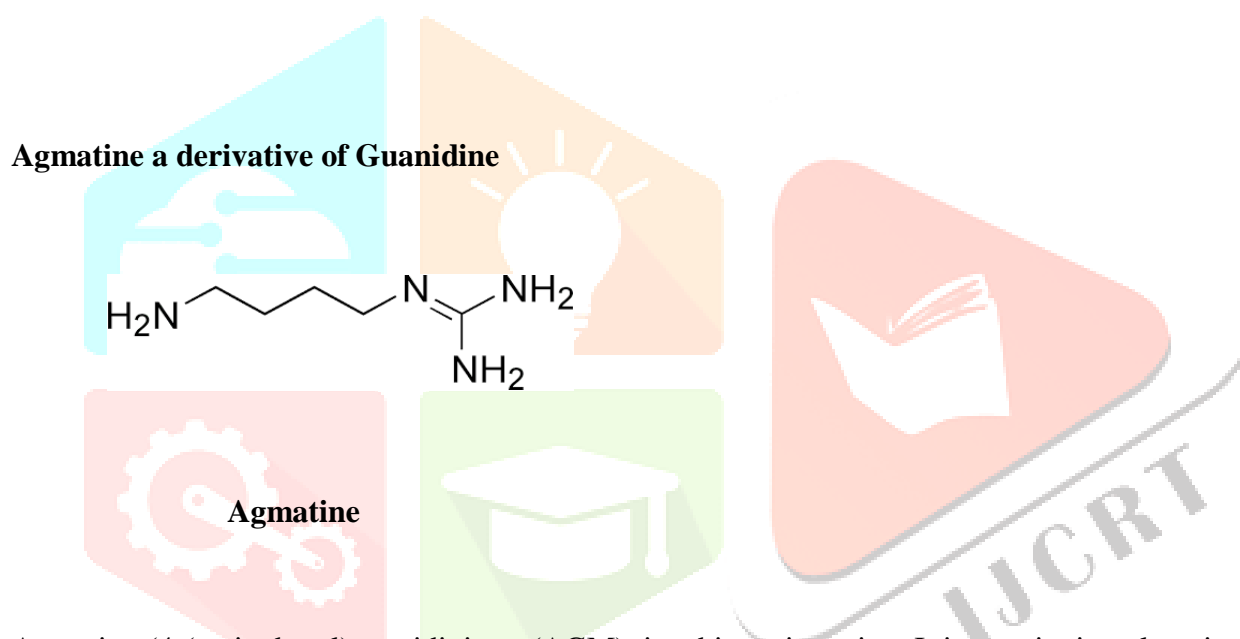
Guanidine, which can be found as the side chain of arginine, is also present in many natural products. Guanidine is one of the most basic organobases and is protonated over a wide pH range. It has drawn much attention recently due to its prominent role in organocatalysis. Over the past decade, new chiral guanidine derivatives have been explored and shown to be excellent catalysts for a variety of enantioselective reactions. Similarly, phase-transfer catalysts (PTC) featuring guanidinium and bisguanidinium salts have succeeded in promoting a variety of enantioselective transformations. As a result of these activities, several reviews on guanidine chemistry have appeared 13.

Compounds containing guanidine as a functional group have attracted considerable synthetic interest due to both the hydrogen bond mediated interactions of guanidinium ions and a wide variety of biological activities. Synthetic guanidines found uses in the engineering of advanced molecular recognition devices, sensors, organic material and phase transfer catalysts. However, that guanidinium ion is one of the most hydrophilic functional group known. Guanidine does not seem to be an ideal scaffolding template on which may be appended side chain specifically oriented to occupy the same relative regions of space as key side

chains of the known ligands⁸. However, polyvalence of guanidine compounds and the variations of their chemical, biochemical and pharmacological activities make them of great importance to the design and development of novel therapeutic agents 14.

The compounds containing guanidine backbone possesses diverse chemical, biochemical and pharmacological properties which makes them important in the design and developments of novel drugs. A series of synthetic analogues and its computational models by a quantitative structure activity relationship (QSAR) program revealed that aminopropylguanidine as well as piperazine-1-carboxamide (PZC) as potent agmatinase inhibitors. Guanidine is synthesized by reacting, amines and cyanamide using suitable reagents at optimum conditions. Guanidine derivatives have antibacterial, antifungal, antitumor activities. Guanidine have effect on CNS, anti-inflammatory agents, inhibitors of $\text{Na}^{(+)}/\text{H}^{(+)}$ exchanger, inhibitors of NO synthase, anti-thrombotic, anti-diabetic and chemotherapeutic agents as well as guanidinium-based transporters and vectors 15.

Guanidino compounds are important metabolic precursors of many substances including ornithine, urea, and creatinine. Agmatine has been mentioned as one of the classical sub- strates of diamine oxidase 16.



Agmatine (4-(aminobutyl)guanidinium, (AGM), is a biogenic amine. It is a cationic polyamine which was first identified in herring sperm in the early 20th century by the German biochemist Albrecht Kossel. Agmatine is synthesized after decarboxylation of L-arginine by arginine decarboxylase (ADC) 17. The biosynthesis of agmatine by ADC, therefore, is dependent upon the availability of L-arginine, which is carried into neurons by specific cationic amino acid transporter and is also a substrate for two other enzymes: arginase and nitric oxide synthase (NOS). Arginase converts L-arginine to ornithine, which enters the urea cycle and NOS catalyses the conversion of L-arginine to nitric oxide (NO) and citrulline. Importantly, NOS is inhibited competitively by agmatine in vitro, an interaction with significant functional consequences regarding the action of agmatine in the brain 18.

In all species, agmatine can be metabolized by hydrolysis to putrescine, the precursor of polyamines spermine and spermidine, by the enzyme agmatinase or oxidized by diamine oxidase to γ -guanidinobutyraldehyde, which is then oxidized to γ -guanidinobutyrate and quickly excreted 19.

Agmatine, ADC and agmatinase have been extensively recognized to be expressed in plants, bacteria, and some invertebrates and are highly preserved in nature 20. Despite until the mid-1990s, they were not believed to be expressed in mammals, it was recently discovered that agmatine, ADC and agmatinase are expressed in mammals 21. Unlike bacterial ADC, which is cytosolic, mammalian ADC resides on mitochondrial membranes, which might explain the lack of results of previous investigation that tried to detect the enzyme in soluble extracts of mammalian tissue 22. Although first detected in brain, agmatine has been quantified in practically all organs of rodents and, in fact, the cerebral concentration of the amine is substantially less than a number of other organs in which agmatine is particularly abundant, mainly stomach, small intestine and aorta 23, 24.

Agmatinase an inhibitor of agmatine

Agmatinase, an ureohydrolase belonging to the arginase family, is widely expressed in mammalian tissues including the brain. Here, it may serve two different functions, the inactivation of the arginine derivative agmatine, a putative neurotransmitter, and the formation of the diamine putrescine. The putative neurotransmitter agmatine is seemingly involved with mental disorders. Therefore, agmatinase may be similarly important for pathogenesis 25. Although, Agmatine is originally identified in the brain as an endogenous neurotransmitter for imidazoline receptors, agmatines effect have mostly been ascribed to inhibition of nitric oxide synthase (NOS) or blockade of glutamate NMDA receptor channels and other ligand-gated cationic channels. Agmatinase is a predominant enzyme and regulates the half life of the agmatine in brain. Therefore, a selective inhibitor of brain agmatinase has been sought 26.

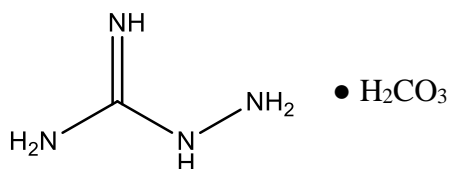
Analogues of agmatine

I. Analogues synthesized for mammalian agmatinase inhibitory activity

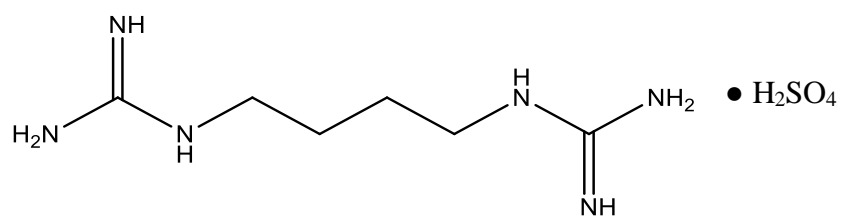
1. Agmatine sulfate



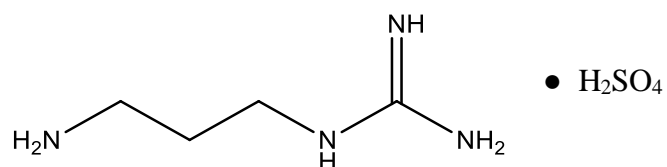
2. Amino guanidine (Aminoguanidine Hemisulfate)



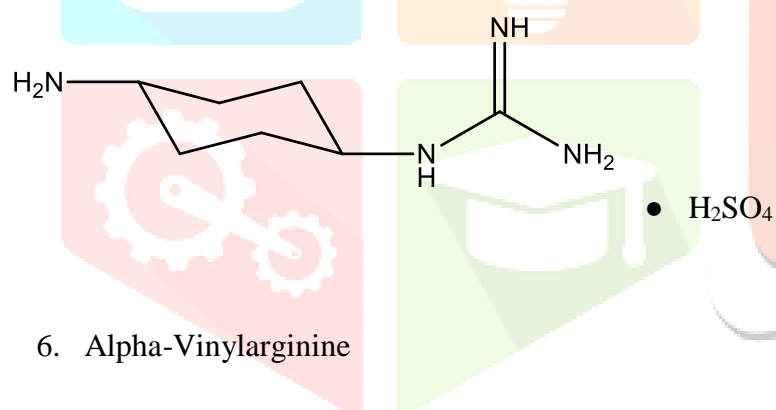
3. Arsenic Sulfate (1,-Diguandinobutane sulphate salt)



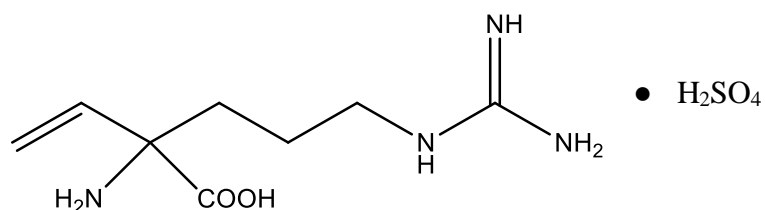
4. 3-Aminopropylguanidine



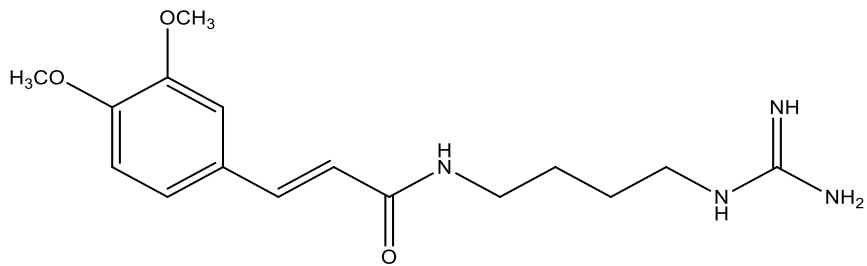
5. Trans 4-aminocyclohexyl guanidine



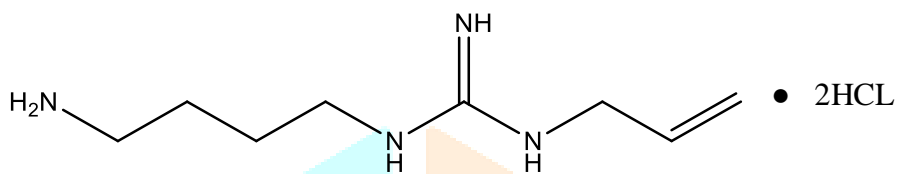
6. Alpha-Vinylarginine



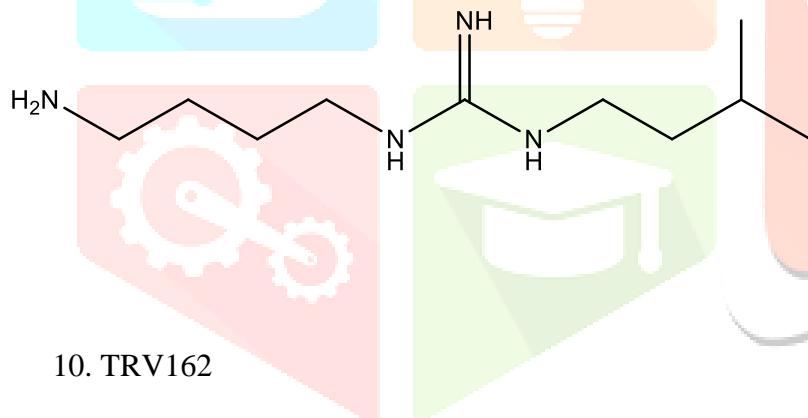
7. CS51



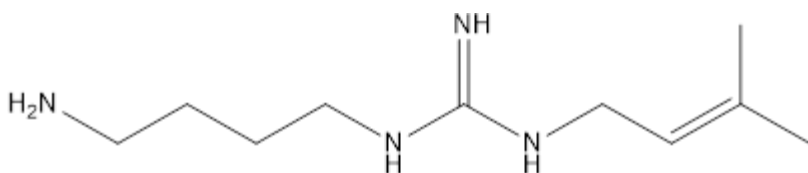
8. R74



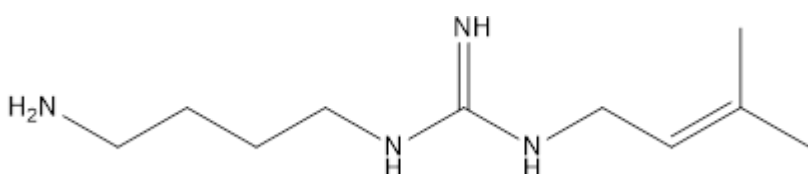
9. TRV187



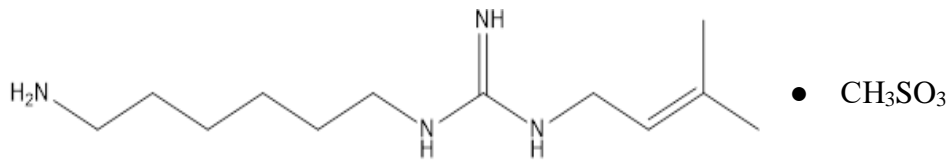
10. TRV162



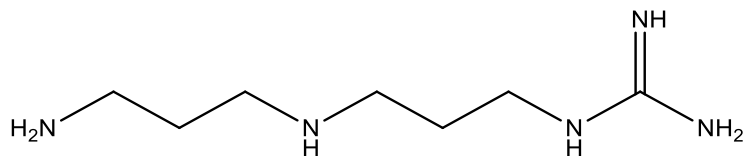
11. G3



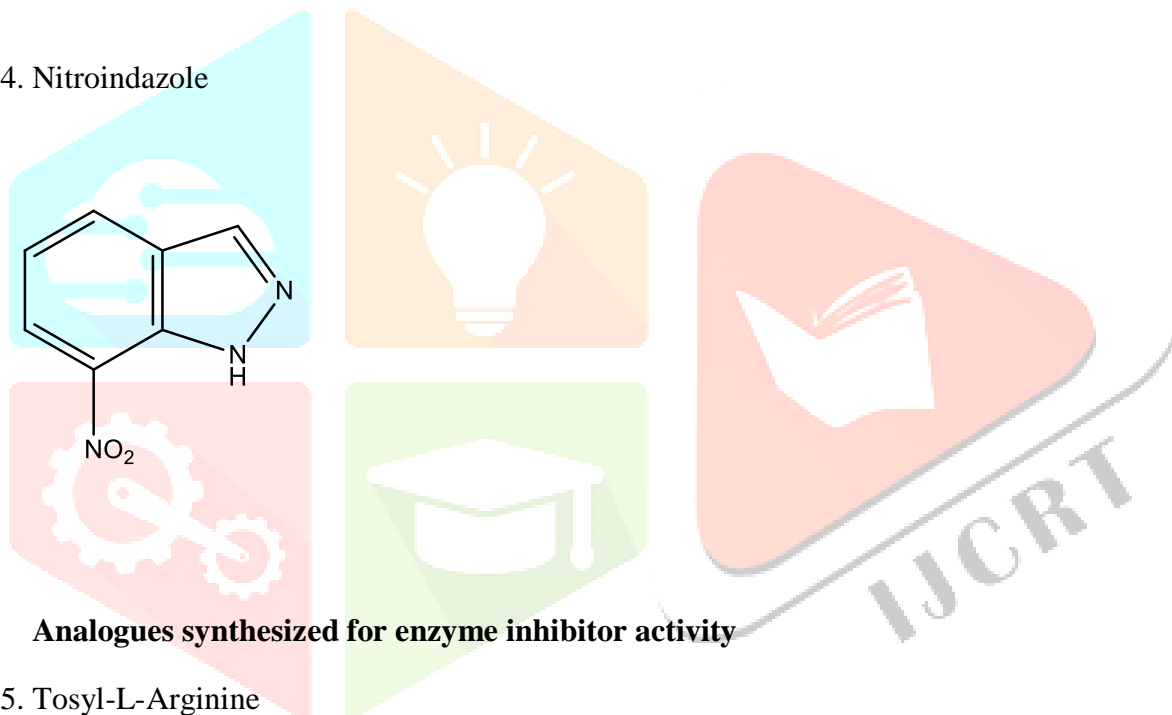
12. RO5



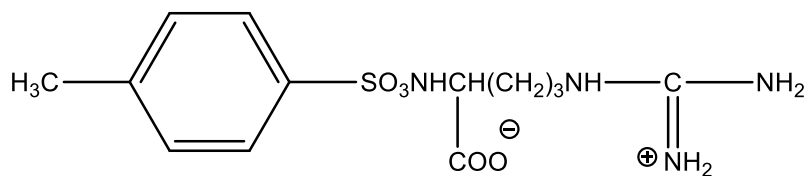
13. Bis (3-(N-iminomethyl)-aminopropyl)amine



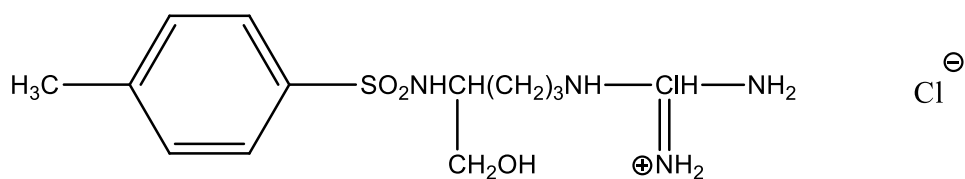
14. Nitroindazole

**II. Analogues synthesized for enzyme inhibitor activity**

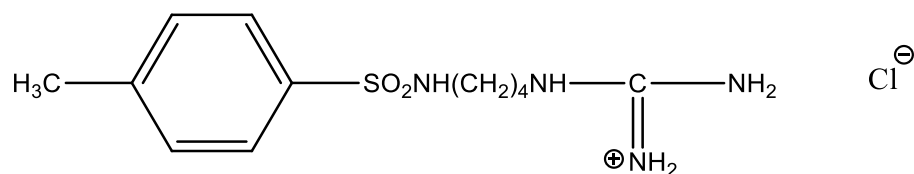
15. Tosyl-L-Arginine



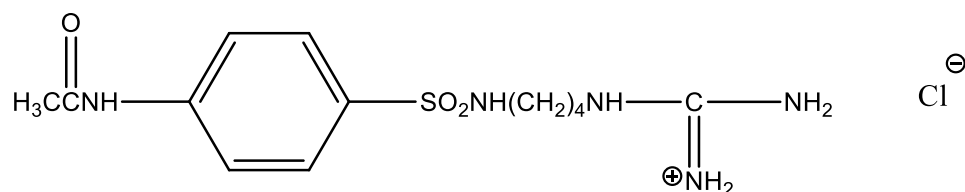
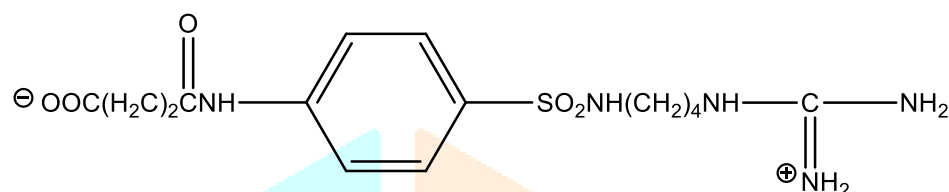
16. Tosyl-L-argininol • HCl



17. Tosylagmatine • HCl



18. p-Acetamidobenzenesulfonylagmatine • HCl

19. p-Succinamidobenzenesulfonylagmatine • H₂O

III. Analogues synthesized using different schemes for analgesic activity

20. N1,N1,2,2-Tetramethyl-N3-[1-(methylamino)-2-nitrovinyl]propane-1,3-diamine

21. N1-[1-(Methylamino)-2-nitrovinyl]butane-1,4-diamine

22. N1-[1-(Methylamino)-2-nitrovinyl]pentane-1,5-diamine

23. N1-[1-(Methylamino)-2-nitrovinyl]hexane-1,6-diamine

24. N-Methyl-2-nitro-N'-pentylethene-1,1-diamine

25. N-Methyl-2-nitro-N'-[4-(piperidin-1-yl)butyl]ethene-1,1-diamine

26. N1-[1-(Methylamino)-2-nitrovinyl]but-2-yne-1,4-diamine

27. N-Methyl-2-nitro-N'-[(pyridin-3-yl)methyl]ethene-1,1-diamine

28. N1-[1-((Pyridin-3-yl)methylamino)-2-nitrovinyl]butane-1,4-diamine

29. N1-[1-((Pyridin-3-yl)methylamino)-2-nitrovinyl]pentane-1,5-diamine

30. Methyl N'-cyano-N-methyl-imidothiocarbamate

31. Methyl N'-cyano-N-[(pyridin-3-yl)methyl]-imidothiocarbamate

32. Methyl N'-cyano-N-isobutyl-imidothiocarbamate

33. Methyl N'-cyano-N-phenylethyl-imidothiocarbamate

34. N''-Cyano-N-[3-(dimethylamino)-2,2-dimethylpropyl]-N'-methylguanidine

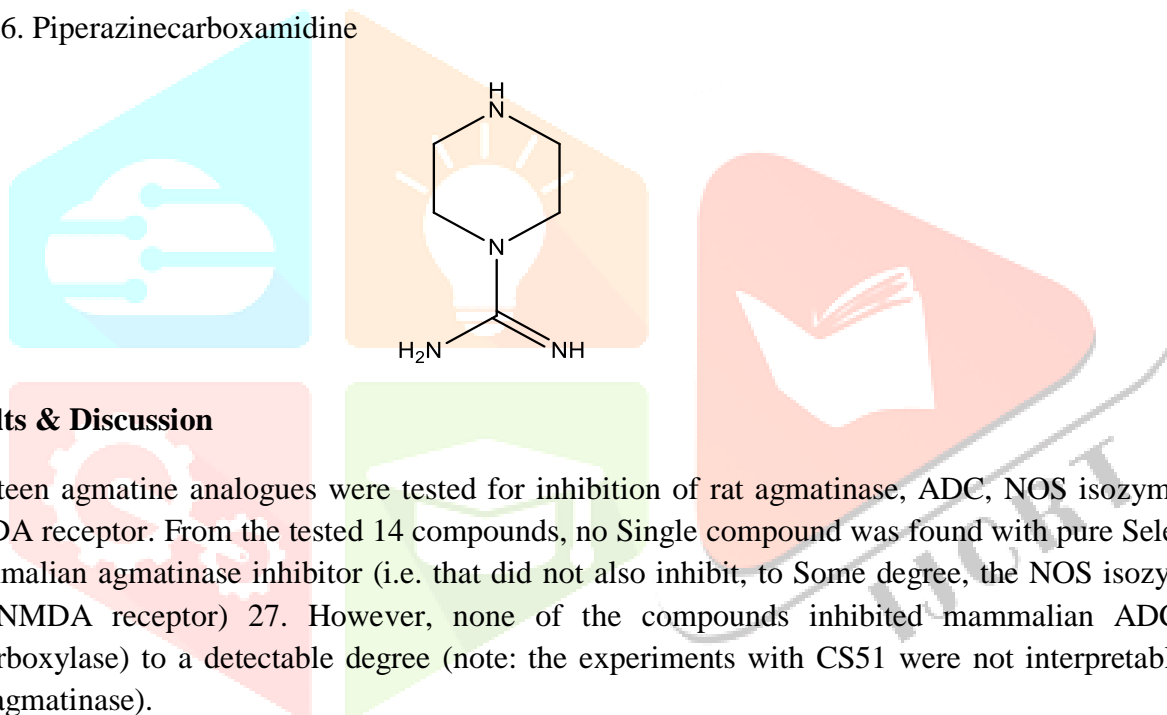
35. N-(4-Aminobutyl)-N''-cyano-N'-methylguanidine

36. N-(5-Aminopentyl)-N''-cyano-N'-methylguanidine

37. N-(6-Aminohexyl)-N''-cyano-N'-methylguanidine
38. N-(7-Aminoheptyl)-N''-cyano-N'-methylguanidine
39. N''-Cyano-N-[3-(dimethylamino)-2,2-dimethylpropyl]-N'-[(pyridin-3-yl)methyl]guanidine
40. N-(6-Aminohexyl)-N''-cyano-N'-[(pyridin-3-yl)methyl]guanidine
41. N-(7-Aminoheptyl)-N''-cyano-N'-[(pyridin-3-yl)methyl]guanidine
42. N-(6-Aminohexyl)-N''-cyano-N'-isobutylguanidine
43. N-(7-Aminoheptyl)-N''-cyano-N'-isobutylguanidine
44. N-(5-Aminopentyl)-N''-cyano-N'-phenethylguanidine
45. N-(7-Aminoheptyl)-N''-cyano-N'-phenethylguanidine

IV. Analogue synthesized for selective agmatinase inhibitor property

46. Piperazinecarboxamidine



results & Discussion

Fourteen agmatine analogues were tested for inhibition of rat agmatinase, ADC, NOS isozymes, and the NMDA receptor. From the tested 14 compounds, no Single compound was found with pure Selectivity as a mammalian agmatinase inhibitor (i.e. that did not also inhibit, to Some degree, the NOS isozymes and/or the NMDA receptor) 27. However, none of the compounds inhibited mammalian ADC (arginine decarboxylase) to a detectable degree (note: the experiments with CS51 were not interpretable for ADC and agmatinase).

None of the compounds examined for this example had absolute Selectivity for mammalian agmatinase. Those compounds that effectively inhibited agmatinase also inhibited NOS isozymes 28.

From the five examples presented in this paper for enzyme inhibitor property, all the various derivatives of the aminobutylguanidine skeleton were shown to inhibit the esteratic activities of trypsin. All the compounds acted as competitive inhibitors, binding to the enzyme is assumed to occur near the catalytic site. As far as specificities are concerned, tosylagmatine and the other agmatine delivatives clearly stand apart from tosyl-L-arginine and tosyl-L-argininol. The p-acetamidobenzenesulfonyl derivative is an appreciably better inhibitor of trypsin than either the tosyl or p-succinamidobenzene- sulfonyl compounds 29.

Derivatives which were synthesized using different schemes for analgesic activity, out of which Compounds N1-[1-(Methylamino)-2-nitrovinyl]hexane-1,6-diamine, N-Methyl-2-nitro-N'-[(pyridin-3-yl)methyl]ethene-1,1-diamine and N''-Cyano-N-[3-(dimethylamino)-2,2-dimethylpropyl]-N'-[(pyridin-3-yl)methyl]guanidine showed notable analgesic activity and good blood-brain barrier permeation profiles 30.

Following extensive screening of analogues, the first evidence that PZC (Piperazinecarboxamidine) might be an agmatinase inhibitor which was clarified by a subsequent QSAR study. That evidence, however, remained grounded in lysate biochemical assays. It was found that derivative of PZC, carboxypiperazine-phosphonate, acts as an NMDRr antagonist.

Like other known neurotransmitters, this neurotransmitter (agmatine) should be under the control of a specific degradation enzyme: namely, agmatinase. Agmatinase activity is somewhat less prominent in the periphery where diamine oxidase (DAO) predominates in the metabolism of peripheral agmatine. Agmatinase is also more substrate-specific than DAO. Other studies have indicated that brain agmatine rises and falls in line with glucocorticoid hormone treatments as well as with specific stressful learning paradigms.

This was an exploratory study in which we found support for PZC as a neuroprotective agent against HI both in vitro and in vivo. The results aligned reasonably with the expectations of an agmatinase inhibitor. However, detailed biochemical studies will be required to definitively prove that PZC's neuroprotection is due to a selective inhibition of agmatinase inhibition. Other future studies are needed to assess PZC's presumed synergy with agmatine and other doses of PZC in other animal species and toxicity models

Conclusion

Guanidino compounds are important metabolic precursors of many substances including ornithine, urea, and creatinine. Agmatine has been mentioned as one of the classical substrates of diamine oxidase. Compounds containing guanidine as a functional group have attracted considerable synthetic interest due to both the hydrogen bond mediated interactions of guanidinium ions and a wide variety of biological activities. 4-aminobutylguanidine acts as neurotransmitter or neuromodulatory agent. From this review we can conclude that Agmatine is responsible for various physiochemical activities in brain as well as in the mammalian body, deficiency of agmatine may lead to various neuromodulatory dysfunctions, hence an appropriate amount of agmatine is required in a mammalian body, as agmatinase is an enzyme which is responsible for the half life of brain agmatine it is necessary to control brain agmatinase hence agmatinase inhibitors plays important role. When the body is unable to produce required amount of agmatine, then it can be externally introduced as agmatinase inhibitor which will ultimately increase the level of agmatine in body, in the present study the derivatives of agmatine which are synthesized till now are reviewed for agmatinase inhibitory property and it can be concluded that none of the derivatives showed selective inhibition of brain agmatinase except one which is piperazine substituted guanidine i.e. PZC (Piperazinecarboxamidine) is expected to show selective inhibition. From the entire study we can suggest that using substituted piperazine the derivatives of agmatine can be synthesized further for better agmatinase inhibition property.

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