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Synthesis Catheptic Inhibitory Studies and Cellular Aspects of Some Heterocyclic Compounds

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ABSTRACT

The named "Combination, catheptic inhibitory investigations and cell parts of some heterocyclic mixtures" portrays the overall strategies for union and portrayal of certain pyrimidines, benzofurans, quinazolines and some furocoumarins dependent on IR, 1H NMR and 13C NMR information and x-beam crystallography. Since heterocyclic mixtures are known to have different organic exercises, the union of these mixtures was attempted as an examination intend to assess their impacts on refined cathepsins B, H and L and HepG2 carcinomal cell lines. The chose chemicals for this examination have been engaged with different sick conditions particularly in disease. The contains five parts and is centered around three perspectives. First viewpoint is the union of some pyrimidine subsidiaries, benzofurans, quinazolines and furocoumarins. The second part of the work manages protein hindrance considers which incorporates disengagement and partition of cathepsins B, H and L. The last viewpoint is to assess the impact of orchestrated mixtures on HepG2 carcinomal cell lines. The substance of every section are summed up underneath. From there on various techniques utilized for the blend of mixtures viable are depicted. Every one of these mixtures were completely described through their IR, 1H NMR, 13C NMR ghostly information and x-beam crystallography of novel mixtures. Distinctive test conventions, for example, compound measures, chemical filtration methods and so forth utilized in catalyst examines are introduced. Impact of various combined mixtures was seen on refined cathepsins, for example, B, H, L and HepG2 carcinomal cell lines. Active examinations were done on inhibitory mixtures to ascertain the Ki esteems. Atomic docking tests are likewise included to relate the in-vitro catalyst restraint concentrates with computationally determined collaboration information.

Keywords – *Heterocyclic Compounds, Synthesis*

INTRODUCTION

Chemicals are normally protein particles that go about as impetus in biochemical responses. The chemical's objective particles called substrate, tie to the dynamic site of compound and get

changed into items through a progression of steps known as the enzymatic system. The typical arrangement of a chemical response can be addressed as:

where:

E = enzyme

S = substrate

E-S = enzyme-substrate complex

E-P = enzyme-product complex

P = product

Michaelis Constant (Km): A protein's Km depicts the substrate fixation at which a large portion of the compound's dynamic locales are involved by substrate. A high Km implies a great deal of substrate should be available to immerse the catalyst, which means the chemical has low liking for the substrate. Then again, a low Km implies just a limited quantity of substrate is expected to soak the chemical, demonstrating a high proclivity for substrate. Graphically, the Km is the substrate focus that gives the protein one-portion of its Vmax.

Protein hindrance is a study of catalyst substrate response impacted by the presence of any natural synthetic or inorganic metal or biosynthetic compound because of their connections with chemical dynamic site. An Enzyme Inhibitor is a particle that ties to protein to influence the action of compound. The control of compound action has a huge assortment of employments. Nowadays the majority of the drug and nutriceutical compounds are advertised as protein inhibitors and such inhibitors display their particular activity by restraining catalysts to address a metabolic irregularity. Not all particles that tight spot to compounds will go about as inhibitors. The mixtures which enact the compound action are called protein activators. An enormous number of usually utilized medications are catalyst inhibitors which incorporate medications to treat HIV, malignant growth and coronary illness. Catalyst inhibitors are likewise utilized in agribusiness as pesticides and herbicides. The activity of chemical inhibitors in medication revelation has become a key way to deal with pharmacology at any drug industry, college research lab or medication research focus.

The cooperation of an inhibitor can prevent a substrate from entering the compound's dynamic site or upset the chemical from catalyzing its response. Inhibitor restricting can be either reversible or irreversible. Irreversible inhibitors generally respond with the catalyst and change it synthetically by development of a covalent bond. These inhibitors change the key amino corrosive deposits that are required for enzymatic action. Interestingly, reversible inhibitors tie non-covalently and various sorts of hindrance are created relying upon whether these inhibitors tie to the compound, the chemical substrate complex, or both.

There are three categories of inhibitors.

- Nonspecific inhibitors
- Irreversible inhibitors
- Reversible inhibitors

Nonspecific Inhibitors: Vague restraint impacts all proteins similarly. This kind of hindrance incorporates any physical or substance changes like temperature, acids and bases, weighty metals, lessening specialists and so on which at last denatures the protein segment of the catalyst and subsequently causes irreversible restraint.

Irreversible Inhibitors: Irreversible inhibitors tie covalently to a protein and cause compound changes to catalyst dynamic site that can't be switched. The irreversible inhibitors have no primary relationship to the substrate. They tie with the dynamic site of the catalyst and obliterate a fundamental practical gathering of dynamic site of the protein. Because of this explanation irreversible inhibitors are utilized for ID of the practical gatherings present at the dynamic site of protein. As irreversible inhibitors normally go about as toxins so they have restricted helpful applications

Reversible inhibitors: These inhibitors tie non-covalently to chemicals that incorporate feeble collaborations like hydrogen securities, hydrophobic connections, and ionic securities and so on Large numbers of these feeble bonds consolidate to create solid and explicit restricting. Rather than irreversible inhibitors, reversible inhibitors for the most part don't go through synthetic responses when bound to the catalyst and can be effortlessly taken out by dialysis or weakening.

OBJECTIVE OF THE STUDY

1. To examination the Synthesis of various heterocyclic mixtures.
2. To examination the Isolation, partition and decontamination of cathepsins B, H and L.

MATERIALS AND METHODS

All the synthetic substances utilized in the analyses were of most noteworthy virtue accessible. Ox-like serum egg whites, dithioerythritol, Fast Garnet GBC were bought from Sigma compound Co., St. Louis, MO, USA.Triton X-100 was secured from Sisco Chemical Laboratories, Bombay. Ammonium sulfate (compound grade) and tris-HCL were bought from Glaxo Laborateries, India. Cysteine.HCl, and 2-mercaptoethanol were provided by Loba Chemical Company, India. The chromatographic media like Sephadex G-100, CM-Sephadex C-50, and DEAE-Sephadex A-50 were bought from Pharmacia Fine Chemicals, Uppsala, Sweden. Different 2-naphthylamide and 4-methoxy-2-naphthylamide substrates like Leu-βNA, BANA, Z-Arg-Arg-4mβNA and Z-Phe-Arg-4mβNA were provided by Bachem Feinchemikalein AG, Bubendorf, Switzerland.

Tissue homogenate was set up by utilizing a decent quality blender cum-processor. IECmake Refrigerated rotator was utilized in different cleansing advances. Amicon ultra filteration cell Model 8050 with YM10 film was utilized to focus different protein tests. Elisa plate peruser 642

was utilized to record the absorbance in the obvious reach. An advanced systronic pH meter model was utilized to quantify the pH estimations of different arrangements. The routine pipetting was finished with programmed pipette. Twofold refined conductivity water was utilized altogether the examinations except if expressed something else. HepG2 cell line was obtained from National Center for Cell Science (NCCS), Pune and refined in α-MEM media (Sigma) enhanced with 10% FBS (Fetal Bovine Serum) (Sigma, US beginning), 10ng/ml epidermal development factor (EGF), 10ng/ml platelet determined development factor (PDGF), glutamine, penicillin/streptomycin and gentamycin. Cells were kept up at a 5% CO2 and 95% air environment at 37°C a humidified CO2 hatchery.

Dissolving focuses were taken in open vessels and are uncorrected. 1H and 13C NMR spectra were recorded on a Brucker 400 MHz instrument (in δ ppm) utilizing TMS as an inner norm. IR spectra (KBr, cm-1) were recorded on a Perkin-Elmer spectrophotometer. Slender layer chromatography (TLC) was performed by utilizing economically arranged silica gel plates and perception was affected with short frequency UV light.

SIGNIFICANCE OF ENZYME INHIBITORS AS DRUG OR MEDICINE

As most of the drug molecules are enzyme inhibitors, so the discovery and improvement in enzyme inhibitors is an active area of research in pharmacology and biochemistry. A medicinal enzyme inhibitor is often judged by its specificity and its potency. A high specificity and potency ensure that a drug will have few side effects and thus low toxicity.

Enzymes maintain the cell homeostasis by way of negative feedback. Other cellular enzyme inhibitors are proteins that specifically bind to and inhibit an enzyme target. This can help to control enzymes that may be damaging to a cell, like proteases or nucleases. A well-characterised example of this is the ribonuclease inhibitor, which binds to ribonucleases by very strong protein–protein interactions. Natural enzyme inhibitors can also be poisons and are used as defences against predators or as ways of killing prey. Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. Many metabolic pathways in the cell are inhibited by metabolites that control enzyme activity through allosteric regulation or substrate inhibition.

A good example is the allosteric regulation of the glycolytic pathway. This catabolic pathway consumes glucose and produces ATP, NADH and pyruvate. Many pesticides used are also enzyme inhibitors. Acetylcholinesterase (AChE) is an enzyme found in animals from insects to humans. It is essential for functioning of nerve cell to break down the neurotransmitter acetylcholine into its constituents, acetate and choline. A large number of AChE inhibitors are used in medicine and agriculture both. Reversible competitive inhibitors like edrophonium, physostigmine and neostigmine are used in the treatment of myasthenia gravis and anaesthesia. The carbamate pesticides are also examples of reversible AChE inhibitors. The organophosphate pesticides such as malathion, parathion, and chlorpyrifos also inhibit irreversibly acetylcholinesterase

Cathepsin B

Cathepsin B (Fig 1.6) (EC 3.4.22.1) has been the most extensively investigated and isolated from various mammalian tissues. Eenzymes from different species do not differ to any great extent. The three-dimensional structure of the enzyme was first obtained in 1991. Cathepsin B is a glycoprotein which has a species-specific carbohydrate residue bound to an Asn residue. The sequence of cathepsin B is known for the enzyme extracted from rat liver, bovine liver and human liver. Cathepsin B is initially synthesized in an inactive form comprising 314 amino acid residues. Amino acids 1-62 at the C-terminal end are essential for correct folding and stabilization of the protease. These 62 residues are subsequently cleaved to liberate the active enzyme. The mature, active cathepsin B enzyme is a polypeptide chain of 252 residues or two chains consisting of 47 and 205 amino acid residues respectively showing close structural homology to papain. About 166 amino acid residues of cathepsin B are equivalent to those in papain. Molecular mass determined by SDS-PAGE for procathepsin B, single chain, heavy chain and light chain are ~42kDa, ~30kDa, ~25kDa and ~5kDa, respectively.

Cathepsin H

Cathepsin H is unique among lysosomal cysteine proteases because it possesses both aminopeptidase and an endopeptidase activity, although the latter activity is much lower than the former activity. The aminopepeptidase activity of cathepsin H is determined by a "mini-chain" that limits the access of substrates to the catalytic center. Sequencing data revealed that in addition to the heavy and light chains, which are typically found in a number of mammalian papain-like cysteine proteases, cathepsin H also contains an octapeptide originating from the propeptide, termed the mini chain. Further studies revealed that the mini chain is disulfide linked to Cys205 of the main body of the enzyme, where it has been suggested to play a role in the aminopeptidase activity of the enzyme. The crystal structure of the porcine enzyme has confirmed the hypothesis and revealed that the mini chain fills the active site cleft of cathepsin H in the region equivalent to the S2 and S3 binding sites in the related endopeptidases. Thereby, the mini chain prevents access of substrates into these non-primed subsites, whereas its C terminal carboxyl group provides the negative charge required for the docking of the positively charged N-terminus of the substrate.

Cathepsin L

Cathepsin L with the highest proteolytic activity in the lysosomes hydrolyzes extracellular matrix proteins such as collagen and elastin more effectively than collagenase and neutrophilic elastase. Cathepsin L differs from cathepsins B and H in that it lacks exopeptidase activity. The molecular weight of active enzyme is ~28 kDa having heavy and light chains of ~24 kDa and ~4 kDa respectively (Fig.1.8). A further difference of cathepsin L is that large amounts (up to 40%) of procathepsin L are secreted relative to cathepsin B and H. Procathepsin L itself has proteolytic activity in the presence of surface materials and this is the first evidence that the proenzyme of a cysteine protease shows catalytic activity. The proregion of procathepsin L is a potent and selective inhibitor of cathepsin L too. A C3-cleaving cysteine protease mainly located at the cell surface shares sequence identities with procathepsin L. Cathepsin L has a broad substrate tolerance i.e. substrates with hydrophobic P2 and P3 residues are preferred.

SYNTHESIS

First of all novel 2-(2-naphthoyl)-6,6-dimethyl-3-aryl-2,3,6,7-tetrahydrobenzofuran4(5H)-one synthesized via one-pot four-component condensation reaction in acetonitrile. In order to achieve the synthesis of our desired product 2-αbromoacetylnaphthalene, dimedone, 4-methylbenzaldehyde and pyridine are chosen as model substrates.

A reaction of 2-α-bromoacetylnaphthalene (1.0 mmol), dimedone (1.0 mmol), 4- substituted benzaldehyde and (1.0 mmol) pyridine was attempted in acetonitrile using Et3N (2.5 mmol) as catalyst under reflux for 12 h. The reaction was quenched using ice cold water and the solid so obtained was subjected to recrystallisation. The product thus obtained 2-(2-naphthoyl)-6, 6-dimethyl-3-(4-substitutedphenyl)-2,3,6,7- tetrahydrobenzofuran-4(5H)-one (62a-62f) were identified by 1H NMR, 13C NMR and IR spectroscopy.

Effect of compounds on cathepsin B

Among the various compounds tested (E)-8-benzylidene-5, 6, 7, 8-tetrahydro-2, 4-diarylquinazolines, 63g was found to be most inhibitory to cathepsin B activity. The activities of cathepsin B were also estimated at varying concentrations of different synthesized compounds. The relationship between the enzyme activity and concentration of quinazolines. Among the various compounds tested, it has been found that cathepsin B activity is inhibited and is affected by the electronegative substituents present in compounds.

Effect of synthesized qinazoline derivatives (63a-63g) on the activity of cathepsin H

Among the various compounds tested 63g was found to be most inhibitory to cathepsin H activity in series of quinazoline derivatives. It has been found that cathepsin H activity is maximally inhibited by the fluoro substituted compound. The activities of cathepsin H were also estimated at varying concentrations of different categories of synthesized compounds. The effect of concentration of (E)-8-benzylidene-5, 6, 7, 8-tetrahydro-2,4-diarylquinazolines on cathepsin H activity. To calculate Ki values of synthesized compounds Lineweaver-Burk plots was drawn in presence of different concentration of substrate. The results show that the compounds inhibited cathepsin H in a noncompetitive manner. The Ki values of most inhibitory compound for cathepsin H has been evaluated as 9.4X10- 10M for (E)-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-2-phenyl-5, 6, 7, 8-tetrahydro quinazoline 63g.

Effect of synthesized qinazoline derivatives (63a-63g) on the activity of cathepsin L

Among the series of compounds 63a-63g, it was found that for cathepsin L flouro substituted quinazolines was most inhibitory [i.e., for (E)-8-(4-fluorobenzylidene)-4- (4-fluorophenyl)-2-phenyl-5,6,7,8-tetrahydroquinazoline 63g with Ki values of 21.4 X10-9M. The calculated Ki values from Lineweaver-Burk equation was shown in Table 4.6. The evaluation of the inhibitory effect of the synthesized compounds on Cathepsins B, H and L on different concentration ranging from 10-4 to 10-9 M. All the synthesized compounds (63a-63g) showed appreciable inhibition. The ihhibition pattern of the synthesized compounds on these enzyme exhibit the order as cathepsin B> cathepsin H> cathepsin L. After establishing the inhibitory action of synthesized compounds on cathepsin B, cathepsin H and cathepsin L experiments were designed to evaluate the type of inhibition and to determine their Ki values. For that, enzyme activity was

evaluated at different substrate concentrations of BANA for cathepsin B, Leu-βNA for cathepsin H and Z-Phe-Arg-4mβNA for cathepsin L respectively in presence and absence of a fixed concentration of inhibitor. The enzyme concentration was kept constant in all the experiments as detailed previously.

CONCLUSION

The study entitled "Synthesis, catheptic inhibitory studies and cellular aspects of some heterocyclic compounds" describes the general methods of synthesis and characterization of some heterocycles like (i) aminopyrimidines and hydroxypyrimidines (ii) Benzofuran derivatives (iii) Quinazoline derivatives and (iv) Furocoumarins. The thesis comprises of the synthesis and characterization of these compounds based on IR, 1H-NMR and 13C spectral data. Synthesized heterocyclic compounds occupy an important position in the effective therapy of number of diseases and disorders. As evident from the reported literature, these compounds are known to possess various biological activities as well as enzyme inhibitory activities so all the synthesized compounds were screened for their activities against cathepsins B, H & L. The studies were further extended for evaluation of their effect on carcinomal HepG2 cell lines. The enzymes were purified and assayed according to the procedures reported in literature. The enzyme cathepsin B obtained was in ~33.92% yield, ~1384.13 fold purification factor and specific activity of enzyme was 10.38 nmoles/min/ml. Cathepsin H was obtained with ~35.33% yield, ~1446.15 fold purification factor and 22.55 nmoles/min/ml specific activity. Cathepsin L was obtained with ~49.96% yield, ~1073.62 fold purification factor and 17.54 nmoles/min/ml specific activities. All the synthesized compounds of pyrimidine, benzofuran, quinazoline and furocoumarin series have been found to possess appreciable catheptic inhibitory potency upto the order of sub nanomolar range. Two of these, benzofurans and quinazolines have been found to have cell proliferating effect with potential use as future anti-inflammatory agents. Pyrimidines and furocoumarins caused cell death with probable potential use as anticancer agents.

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