CHAPTER – 1
AN OVERVIEW OF ANTIBIOTIC DRUGS

1.1 Introduction

An antibiotic is an agent that either kills or inhibits the growth of a microorganism. The term antibiotic was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Waksman 1947). This definition excluded substances that kill bacteria but that are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds such as the sulfonamides. Many antibacterial compounds are relatively small molecules with a molecular weight of less than 2000 atomic mass units.

With advances in medicinal chemistry, most modern antibacterials are semisynthetic modifications of various natural compounds (Von Nussbaum et al 2006). These include, for example, the beta-lactam antibacterios, which include the penicillins (produced by fungi in the genus Penicillium), the cephalosporins, and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterials—for example, the sulfonamides, the quinolones, and the oxazolidinones—are produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic, and...
synthetic. Another classification system is based on biological activity; in this classification, antibacterials are divided into two broad groups according to their biological effect on microorganisms: Bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth.

1.1.1 Classes

Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes (Calderon et al 2007). Those that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane (polymyxins), or interfere with essential bacterial enzymes (rifamycins, lipiarmycins, quinolones, and sulfonamides) have bactericidal activities. Those that target protein synthesis (macrolides, lincosamides and tetracyclines) are usually bacteriostatic (with the exception of bactericidal aminoglycosides) (Finberg et al 2004). Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria. Following a 40-year hiatus in discovering new classes of antibacterial compounds, four new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycyclcyclines (such as tigecycline), oxazolidinones (such as linezolid), and lipiarmycins (such as fidaxomicin) (Cunha 2009 and Srivastava et al 2011).
1.1.2 Side Effects

Antibiotics are screened for any negative effects on humans or other mammals before approval for clinical use, and are usually considered safe and most are well-tolerated. However, some antibiotics have been associated with a range of adverse side effects (Slama et al 2005). Side-effects range from mild to very serious depending on the antibiotics used, the microbial organisms targeted, and the individual patient. Safety profiles of newer drugs are often not as well-established as for those that have a long history of use. Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Common side-effects include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as Clostridium difficile. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus Candida in the vulvo-vaginal area (Pirotta M V and Garland S M 2006). Additional side-effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid. Some scientists have hypothesized that the indiscriminate use of antibiotics alter the host microbiota and this has been associated with chronic disease (Thacker D James 2012).

1.2 Antibiotic Resistance

The emergence of resistance of bacteria to antibiotics is a common phenomenon. Emergence of resistance often reflects evolutionary processes that
take place during antibiotic therapy. The antibiotic treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant bacteria, while growth of susceptible bacteria is inhibited by the drug (Levy 1994). For example, antibacterial selection for strains having previously acquired antibacterial-resistance genes was demonstrated in 1943 by the Luria–Delbrück experiment (Luria and Delbruck 1943). Antibiotics such as penicillin and erythromycin, which used to have a high efficacy against many bacterial species and strains, have become less effective, due to the increased resistance of many bacterial strains.

Resistance may take the form of biodegradation of pharmaceuticals, such as sulfamethazine-degrading soil bacteria introduced to sulfamethazine through medicated pig feces (Topp et al 2013). The survival of bacteria often results from an inheritable resistance (Witte 2004), but the growth of resistance to antibacterials also occurs through horizontal gene transfer. Horizontal transfer is more likely to happen in locations of frequent antibiotic use (Dyer, Betsey Dexter 2003).

Antibacterial resistance may impose a biological cost, thereby reducing fitness of resistant strains, which can limit the spread of antibacterial-resistant bacteria, for example, in the absence of antibacterial compounds. Additional mutations, however, may compensate for this fitness cost and can aid the survival of these bacteria (Andersson 2006). Paleontological data show that both
antibiotics and antibiotic resistance are ancient compounds and mechanisms (D’Costa et al 2011). Useful antibiotic targets are those for which mutations negatively impact bacterial reproduction or viability (Gladki et al 2013).

Several molecular mechanisms of antibacterial resistance exist. Intrinsic antibacterial resistance may be part of the genetic makeup of bacterial strains (Alekshun and Levy 2007). For example, an antibiotic target may be absent from the bacterial genome. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. Antibacterial-producing bacteria have evolved resistance mechanisms that have been shown to be similar to, and may have been transferred to, antibacterial-resistant strains (Marshall et al 1998 and Nikaido 2009). The spread of antibacterial resistance often occurs through vertical transmission of mutations during growth and by genetic recombination of DNA by horizontal genetic exchange. For instance, antibacterial resistance genes can be exchanged between different bacterial strains or species via plasmids that carry these resistance genes. Plasmids that carry several different resistance genes can confer resistance to multiple antibacterials. Cross-resistance to several antibacterials may also occur when a resistance mechanism encoded by a single gene conveys resistance to more than one antibacterial compound (Baker-Austin et al 2006).

Antibacterial-resistant strains and species, sometimes referred to as "superbugs", now contribute to the emergence of diseases that are for a while well-controlled. For example, emergent bacterial strains causing tuberculosis
(TB) that are resistant to previously effective antibacterial treatments pose many therapeutic challenges. Every year, nearly half a million new cases of multidrug-resistant tuberculosis (MDR-TB) are estimated to occur worldwide. For example, NDM-1 is a newly identified enzyme conveying bacterial resistance to a broad range of beta-lactam antibacterials. The United Kingdom's Health Protection Agency has stated that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections."

1.2.1 Status of new Antibiotics Development

In a policy report released by the Infectious Disease Society of America (IDSA) on April 2013, IDSA expressed grave concern over the weak pipeline of antibiotics to combat the growing ability of bacteria, especially the Gram-negative bacilli (GNB), to develop resistance to antibiotics. Since 2009, only 2 new antibiotics are approved in United States, and the number of new antibiotics annually approved for marketing continues to decline. The report could identify only seven antibiotics currently in phase 2 or phase 3 clinical trials to treat the GNB, which includes E. coli, Salmonella, Shigella, and the Enterobacteriaceae bacteria, and these drugs do not address the entire spectrum of the resistance developed by those bacteria (Boucher et al 2013).

The IDSA’s prognosis for sustainable R&D infrastructure for antibiotics development will depend upon clarification of FDA regulatory clinical trial guidance that would facilitate the speedy approval of new drugs, and the
appropriate economic incentives for the pharmaceuticals companies to invest in this endeavor. On 12 December 2013, the Antibiotic Development to Advance Patient Treatment (ADAPT) Act of 2013 was introduced in the U.S. Congress. The ADAPT Act aims to fast track the drug development in order to combat the growing public health threat of 'superbugs'. Under this Act, FDA can approve antibiotics and antifungals needed for life-threatening infections based on data from smaller clinical trials. The CDC will reinforce the monitoring of the use of antibiotics that treat serious and life-threatening infections and the emerging resistance, and make the data publicly available. The FDA antibiotics labeling process, 'Susceptibility Test Interpretive Criteria for Microbial Organisms' or 'breakpoints' is also streamlined to allow the most up-to-date and cutting-edge data available to healthcare professionals under the new Act. Congress has been urged in 2014 from several parties to aid the development of new drugs via bills such as ADAPT. Allan Coukell, director of drugs and medical devices at The Pew Charitable Trusts, testified in from of the House Committee, in a statement published by Reuters, that "By allowing drug developers to rely on smaller datasets, and clarifying FDA's authority to tolerate a higher level of uncertainty for these drugs when making a risk/benefit calculation, ADAPT would make the clinical trials more feasible."

1.3 Antibiotics Drug

Antibiotics are a group of medicines that are used to treat infections caused by bacteria and certain parasites. They are sometimes called
antibacterials. Antibiotics can be taken by mouth as liquids, tablets, or capsules, or they can be given by injection. Usually, people who need to have an antibiotic by injection are in hospital because they have a severe infection. Antibiotics are also available as creams, ointments, or lotions to apply to the skin to treat certain skin infections. It is important to remember that antibiotics only work against infections that are caused by bacteria and certain parasites. They do not work against infections that are caused by viruses (for example, the common cold or flu), or fungi (for example, thrush in the mouth or vagina), or fungal infections of the skin.

Occasionally, a viral infection or minor bacterial infection develops into a more serious secondary bacterial infection. There are various antibiotics available and they come in various different brand names. Antibiotics are usually grouped together based on how they work. Each type of antibiotic only works against certain types of bacteria or parasites. This is why different antibiotics are used to treat different types of infection.

1.3.1 Types of Antibiotics

Although there are well over 100 antibiotics, the majority come from only a few types of drugs. These are the main classes of antibiotics.

- Penicillins such as penicillin and amoxicillin
- Cephalosporins such as cephalexin (Keflex)
- Macrolides such as erythromycin (E-Mycin), clarithromycin (Biaxin), and azithromycin (Zithromax)
• Fluoroquinolones such as ciprofloxacin (Cipro), levofloxacin (Levaquin), and ofloxacin (Floxin)
• Sulfonamides such as co-trimoxazole (Bactrim) and trimethoprim (Proloprim)
• Tetracyclines such as tetracycline (Sumycin, Panmycin) and doxycycline (Vibramycin)
• Aminoglycosides such as gentamicin (Garamycin) and tobramycin (Tobrex)

Most antibiotics have two names, the trade or brand name, created by the drug company that manufactures the drug, and a generic name, based on the antibiotic's chemical structure or chemical class. Trade names such as Keflex and Zithromax are capitalized. Generics such as cephalexin and azithromycin are not capitalized.

Each antibiotic is effective only for certain types of infections, and your doctor is best able to compare your needs with the available medicines. Also, a person may have allergies that eliminate a class of antibiotic from consideration, such as a penicillin allergy preventing your doctor from prescribing amoxicillin.

In most cases of antibiotic use, a doctor must choose an antibiotic based on the most likely cause of the infection. For example, if you have an earache, the doctor knows what kinds of bacteria cause most ear infections. In another example, a few bacteria cause about 90% of pneumonias in previously healthy
people. If you are diagnosed with pneumonia, the doctor will choose an antibiotic that will kill these bacteria.

Other factors may be considered when choosing an antibiotic. Medication cost, dosing schedule, and common side effects are often taken into account. Patterns of infection in your community may be considered also. In some cases, laboratory tests may be used to help a doctor make an antibiotic choice. Special strains of the bacteria such as Gram stains, can be used to identify bacteria under the microscope and may help narrow down which species of bacteria is causing infection. Certain bacterial species will take a stain, and others will not. Cultures may also be obtained. In this technique, a bacterial sample from your infection is allowed to grow in a laboratory. The way bacteria grow or what they look like when they grow can help to identify the bacterial species. Cultures may also be tested to determine antibiotic sensitivities. A sensitivity list is the roster of antibiotics that kill a particular bacterial type. This list can be used to double check that you are taking the right antibiotic.

1.3.2 Antibiotics work

Some antibiotics work by killing bacteria or the parasite. This is often done by interfering with the structure of the cell wall of the bacterium or parasite. Some work by stopping bacteria or the parasite from multiplying. Antibiotics are normally only prescribed for more serious bacterial infections, and for some parasitic infections. Most common infections are caused by viruses, when an antibiotic will not be of use. Even if you have a mild bacterial infection, the
immune system can clear most bacterial infections. For example, antibiotics usually do little to speed up recovery of bronchitis, or most ear, nose, and throat infections that are caused by bacteria. So, do not be surprised if a doctor does not recommend an antibiotic for conditions caused by viruses or non-bacterial infections, or even for a mild bacterial infection. However, you do need antibiotics if you have certain serious infections caused by bacteria such as meningitis or pneumonia. In these situations, antibiotics are often life-saving.

When you are ill, doctors are skilled at checking you over to rule out serious illness, and to advise if an antibiotic is needed. Antibiotics can also be prescribed to treat acne - a less serious condition. For acne, antibiotics can be taken orally or applied directly to the skin.

1.3.3 Antibiotic Used

The choice of antibiotic mainly depends on which infection you have and the bacterium or parasite your doctor thinks is causing your infection. This is because each antibiotic is effective only against certain bacteria and parasites. For example, if you have pneumonia, the doctor knows what kinds of bacteria typically cause most cases of pneumonia. He or she will choose the antibiotic that best combats those kinds of bacteria. There are other factors that influence the choice of an antibiotics. These include: how severe the infection is, how well your kidneys and liver are working, dosing schedule, other medications you may be taking, common side-effects, a history of having an allergy to a certain type
of antibiotic, or if you are pregnant or breast-feeding. Even there are a number of antibiotics that are thought to be safe to take.

It is important to take antibiotics in the correct way. If you do not, this may reduce how well they work. For example, some antibiotics need to be taken with food and others should be taken on an empty stomach. If you do not take your antibiotics in the right way it will affect their absorption (how much gets into the body), and therefore they may not work as well. So, follow the instructions as given by your doctor and on the leaflet that comes with the antibiotic you are prescribed. Always take the entire course of antibiotics as directed by your doctor. Even though you may feel better before your medicine is entirely gone, follow through and take the entire course. This is important for your healing. If an antibiotic is stopped in mid-course, bacteria may be partially treated and not completely killed. Bacteria may then become resistant to that antibiotic. Overuse of antibiotics has led to some bacteria mutating and becoming resistant to some antibiotics, which may then not work when really needed. For example, Meticillin-Resistant Staphylococcus Aureus (MRSA) is a bacterium that has become resistant to many different antibiotics and is difficult to treat.

1.3.4 Length of treatment

The length of treatment varies a lot. It depends on what kind of infection you have, how severe it is and how quickly you get better after starting treatment. Treatment can be for just a few days (urinary tract infection - water infection),
one or two weeks (pneumonia), a few months (bone infections), or for many months (acne).

### 1.4 Samples

In the present work, the antibiotic drugs namely (i) Pregabalin, (ii) Chlorzoxazone, (iii) Metronidazole, (iv) Cephalexin, and (v) Leucine are chosen for investigation. The physico-chemical properties of the drugs are given in Table 1.1. A brief description of the above drugs is given below.

**(i) Pregabalin**

Pregabalin is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults (Benker et al 2006). It has also been found effective for generalized anxiety disorder and is (as of 2007) approved for this use in the European Union and Russia. It is effective at treating some causes of chronic pain such as fibromyalgia but not others. It is considered to have a low potential for abuse, and a limited dependence liability if misused, but is classified as a Schedule V drug in the U.S. It was designed as a more potent successor to gabapentin.

Pregabalin is marketed by Pfizer under the trade name Lyrica. Pfizer described in an SEC filing that the drug could be used to treat epilepsy, postherpetic neuralgia, diabetic peripheral neuropathy and fibromyalgia. Lyrica was promoted for other uses which had not been approved by medical regulators up until 2009. For this practice, with 3 other drugs, Pfizer was fined a record amount of $2.3 billion by the Department of Justice.
Table 1.1 Physico-chemical properties

<table>
<thead>
<tr>
<th>Name of the drug</th>
<th>Pregabalin</th>
<th>Chlorzoxazone</th>
<th>Metronidazole</th>
<th>Cephalexin</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Formula</strong></td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;ClNO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;N&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;S</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;NO&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>159.2</td>
<td>169.56</td>
<td>171.15</td>
<td>347.39</td>
<td>131.17</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
<td>White to off-white crystalline solid</td>
<td>White to Off-White Solid</td>
<td>white to slightly yellow crystalline powder</td>
<td>White cryst. powder</td>
<td>Crystalline</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Freely soluble in water and both basic and acidic solutions</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O: soluble 0.1 g/100 mL</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O: soluble &lt;0.1 g/100 mL at 20 ºC</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;OH 1 M: 50 mg/mL, clear, yellow</td>
<td>Water soluble</td>
</tr>
<tr>
<td><strong>Prescribed storage condition</strong></td>
<td>should be stored at room temperature, between 15 C to 30 C</td>
<td>−20ºC</td>
<td>2ºC - 8ºC</td>
<td>2ºC - 8ºC</td>
<td>-20ºC</td>
</tr>
</tbody>
</table>
The European Federation of Neurological Societies recommends pregabalin as a first line agent for the treatment of pain associated with diabetic neuropathy, post-herpetic neuralgia, and central neuropathic pain (Attal N et al 2010). Other first line agents, including gabapentin and tricyclic antidepressants, are given equal weight as first line agents, and unlike pregabalin, are available as inexpensive generics (Finnerup et al 2010). It is not recommended for certain other types of neuropathic pain such as pain associated with trigeminal neuralgia or HIV infection and its use in cancer-associated neuropathic pain is controversial (Bennett 2013). There is no evidence for its use in the prevention of migraines and gabapentin (Linde et al 2013). It has been examined for the prevention of post-surgical chronic pain, but its utility for this purpose is controversial (Clarke 2012 and Chaparro et al 2013).

(ii) Chlorzoxazone

Chlorzoxazone is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders (Bailey 1995). It is a centrally acting muscle relaxant used to treat muscle spasm and the resulting pain or discomfort. It acts on the spinal cord by depressing reflexes. It is sold as Muscol or Parafon Forte, a combination of chlorzoxazone and acetaminophen (Paracetamol). The possible side effects include dizziness, light-headedness, malaise, nausea, vomiting, and liver dysfunction. It can also be administered for acute pain in general and for tension headache (muscle contraction headache). However, this
medicine does not take the place of rest, exercise, physical therapy, or other treatments that your doctor may recommend for your medical condition.

All medicines may cause side effects, but many people have no, or minor, side effects. Check with your doctor if any of these most COMMON side effects persist or become bothersome: Dizziness; drowsiness; general body discomfort; light-headedness; nausea; nervousness; over-stimulation. Seek medical attention right away if any of these SEVERE side effects occur: Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue; unusual hoarseness); black, tarry stools; bloody or coffee ground-like vomit; fever; symptoms of liver problems (eg, loss of appetite; unusual nausea, vomiting, or tiredness; stomach pain; dark urine; pale stools; yellowing of the skin or eyes); weight loss.

(iii) Metronidazole

Metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa. It is antibacterial against anaerobic organisms, an amoebicide, and an antiprotozoal. It is the drug of choice for first episodes of mild-to-moderate Clostridium difficile infection (Cohen et al 2010). It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

Metronidazole is primarily used to treat: bacterial vaginosis, pelvic inflammatory disease (along with other antibacterials like ceftriaxone), pseudomembranous colitis, aspiration pneumonia, rosacea (topical), fungating
wounds (topical), intra-abdominal infections, lung abscess, gingivitis, amoebiasis, giardiasis, trichomonia, and infections caused by susceptible anaerobic organisms such as Bacteroides, Fusobacterium, Clostridium, Peptostreptococcus, and Prevotella species. It is also often used to eradicate Helicobacter pylori along with other drugs and to prevent infection in people recovering from surgery (Rossi 2013).

(iv) Cephalexin

Cephalexin (INN) or more commonly cephalexin is a first-generation cephalosporin antibiotic introduced in 1967 by Eli Lilly and Company (Sweetman et al. 2006 and Sneader 2005). It is an orally administered agent with a similar antimicrobial spectrum to the intravenous agents cefalotin and cefazolin. It was first marketed as Keflex (Lilly), and is marketed under several other trade names. As of 2008, cefalexin was the most popular cephalosporin antibiotic in the United States, with more than 25 million prescriptions of its generic versions alone, for US$255 million in sales (though less popular than two other antibiotics, amoxicillin and azithromycin, each with 50 million prescriptions.

Cefalexin is used to treat a number of infections including: otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulitis, and urinary tract infections. It may be used to prevent bacterial endocarditis.

In addition to being a rational first-line treatment for cellulitis, it is a useful alternative to penicillin’s in patients with penicillin hypersensitivity. In
patients with mild or questionable history of penicillin allergy, cephalosporin’s are now thought to be relatively safe (Pichichero 2003). Caution should always be taken when prescribing cephalosporin’s to those with strong history of true penicillin hypersensitivity, however, because cefalexin and other first-generation cephalosporin’s are known to have a modest cross-allergy in patients with penicillin hypersensitivity.

*(v) Leucine*

Leucine (abbreviated as Leu or L) is a branched-chain α-amino acid. Leucine is classified as a hydrophobic amino acid due to its aliphatic isobutyl side chain. It is encoded by six codons (UUA, UUG, CUU, CUC, CUA, and CUG) and is a major component of the subunits in ferritin, astacin, and other 'buffer' proteins. Leucine is an essential amino acid, meaning that the human body cannot synthesize it, and it therefore must be ingested.

Leucine is one of the three Branched Chain Amino Acids and sometimes referred to as the 'main' amino acid due to the most popular benefit of BCAAs (muscle building) being mostly due to leucine. Leucine is an activator of the protein known as MTOR, which then induces muscle protein synthesis via S6K; the other two BCAAs may also activate MTOR, but are much weaker than leucine in doing so (and as such, 5g of leucine will be more effective than 5g mixed BCAAs). The leucine metabolite, HMB, is also weaker than leucine at inducing muscle protein synthesis despite being more effective at preserving lean mass from breakdown. Leucine is a tad different from the other two BCAAs
Isoleucine and Valine as leucine seems to have a fair bit of testing on the amino acid in isolation rather than in a BCAA mixture, whereas the other two BCAAs are not as well studied.

The studies assessing leucine mostly look at muscle protein synthesis when additional leucine is added to the diet or to a test meal, and it appears that leucine is able to reliably increase muscle protein synthesis after test meals. The interactions of leucine on glucose are not clear, to be honest. Leucine possesses both blood sugar reducing properties (can release insulin from the pancreas, can directly stimulate glucose uptake into a cell without insulin) but also the opposite (via stimulating S6K, it can inhibit insulin-stimulated glucose uptake). In a cell culture, leucine stimulates glucose uptake for up to 45 minutes and then hinders itself while in living systems acute doses of leucine do not appear to do anything remarkable (some limited evidence that leucine can be rehabilitative in diabetes, but this is preliminary). Isoleucine is a more potent hypoglycemic agent, but to less inhibition of its own actions.