CHAPTER 1

INTRODUCTION

Anxiety is a normal functional state in mammals often conducive to environmental awareness and survival. It is a complex of subjective feelings and characteristic behaviors. The subjective feelings consist of tension, apprehension, fear, worry, and difficulty with thinking or concentrating. These feelings are usually accompanied by behavioral signs and symptoms of trembling, tremors, muscle tension, restlessness, and fatigue with autonomic hyperactivity in the respiratory, cardiovascular, urinary, and gastrointestinal (GI) systems. Such signs and symptoms of anxiety can be altogether normal, appropriate, and beneficial responses to threatening or tragic situations. But anxiety can take on harmful and medically meaningful dimensions when it is inappropriate to the situation or functionally disabling. Thus, the need for diagnosis and treatment is a function not only of the symptoms but of their intensity, duration, and the degree to which they interfere with other activities. (Smith, 1992).

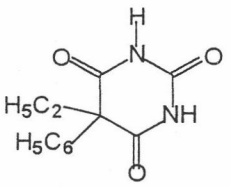
Anxiety can also be a common concomitant of many organic diseases, such as hypoglycemia, anemia, vitamin B12 deficiency, hyperthyroidism, coronary heart disease, and mitral valve prolapse. It may also be a prominent symptom in many psychiatric patients, especiallly those with personality disorders, mood (affective) disorders,

or schizophrenia. A wide variety of drugs including, caffeine, theophylline, ephedrine, amphetamines, cocaine, thyroid hormones, digitalis, imipramine, indomethacin, baclofen, levodopa, propranolol, as well as rebound or withdrawal from alcohol or benzodiazepine use can produce or aggravate anxiety symtoms (Smith, 1992).

Drugs used in the therapy of disorders of feeling, thoughts, and behaviors are now usually classified according to their uses in the therapy of identified diseases or conditions. The antianxiety drugs are drugs that can antagonize and relieve anxiety in many of the syndromes listed earlier, as well as the anxiety of everyday life; however, their use in therapy is best limited to administration for relatively short periods of time usually a few days to a month, and to those situations and conditions in which such short-term therapy is useful, such as preoperatively, for acute grief reactions that impair functioning, or for insomnia of brief duration due to worry over short-lived transient external events.

Many classes of drugs are used for the treatment of anxiety (Baldessarini,1990): such as

- Barbiturate group: e.g. phenobarbital (I).
- Propanediol carbamates: e.g. meprobamate (II).
- Antihistamines: e.g. hydroxyzine (III).
- β-Adrenergic antagonists: e.g. propanolol (IV).
- Benzodiazepines and non-benzodiazepines.



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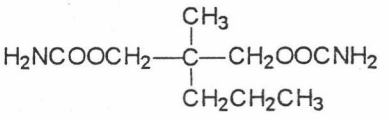

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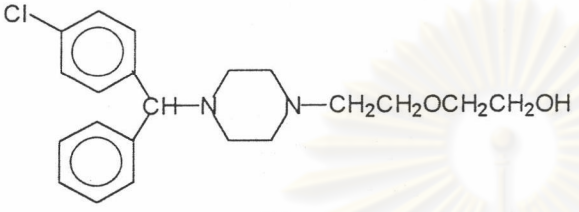

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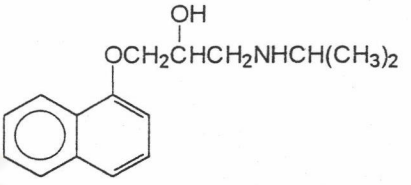


(IV)

Man has sought chemical agents to modify the effects of stress and the feelings of discomfort, tension, anxiety, and dysphoria throughout recorded history. Many of these efforts have led to the development of agents that are often classed as sedative, and the single most widely used of these is one of the oldest-ethanol. In the last century, bromide salts and the barbiturates were introduced into medical practice as sedatives, along with compounds similar in effect to alcohol, including paraldehyde and chloral hydrate. By the 1930s it became apparent that bromides had cumulative toxic effects on the CNS, and their use in medical practice has largely disappeared. Throughout the early decades of this century, the barbiturates were the dominant antianxiety agents in medical practice; however, by the 1950s, there was concern with their propensity to induce tolerance, sometimes followed by physical dependence and potentially lethal reactions during withdrawal. These problems strongly colored professional and popular attitudes about sedatives and encouraged the search for safer agents. This experience probably contributed to the use

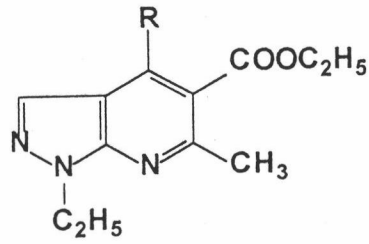
of new terms that emphasize putative dissimilarities of newer agents from the barbiturates and related sedatives. Studies of derivatives of aliphatic polyalcohols led to the development of mephenesin, the o-methyl-phenyl derivative of propanetriol; this agent was found to have muscle relaxant and sedative properties, but was impractically short acting. Its chemical modifications led directly to the introduction of the propanediol carbamates (meprobamate and congeners) in the early 1950s, along with a variety of other analogs of barbiturates or derivatives of higher alcohols. Throughout the 1950s, despite the popularity of some of these compounds for daytime sedation or for hypnotic effects, an increasing awareness developed that they shared many of the undesirable properties of barbiturates. These included an unclear separation between their useful antianxiety effects and excessive sedation and an impressive propensity to cause physical dependence and severe acute intoxication on overdosage. This set the scene for the discovery of chlordiazepoxide in the late 1950s and the introduction of more than a dozen benzodiazepine congeners since that time.

The benzodiazepines have become much-used drug in several therapeutic areas during 20 or more years of clinical experiences. They are used as anxiolytic, anticonvulsant, sedative and muscle relaxant. However, recently it was found that benzodiazepines can induce memory deficits (Kulkarni and Sharma,1990; Brioni,1993). Over a period of 10 years, a large number of benzodiazepine molecules were synthesized and many were marketed for their different therapeutic uses. A major advance in the understanding of the mechanism of action of

benzodiazepines was the discovery of receptors for benzodiazepines, which was characterized in 1977 by radioligand binding studies using ^{3}H-diazepam (Braestrup and Nielsen,1982; Braestrup *et al*, 1984). Benzodiazepine receptors are localized in the brain (such as on limbic system, cortical association areas, reticular or celebellar system) and peripheral tissues (such as heart and skeleton muscle). The description of high-affinity, saturable binding sites for the benzodiazepines raised the possibility that there might exist an endogeneous ligands for the benzodiazepine receptors that could be either anxiolytic or anxiogenic (anxiety inducing). It has been proposed that agonist differential selectivity for benzodiazepine. At least three receptor subtypes, ω1, ω2 and ω3 may account for the varied pharmacological action of known anxiolytics which own their activity to this mechanism of action. The designation ω1, ω2 and ω3 for receptor subtypes are replacing the use of BZ1, BZ2 and BZ3 (receptor at peripheral sites), respectively. This classification and widely accepted proposal has stimulated renewed interest in the discovery of novel anxiolytics in which the pharmacological profile is fine-tuned by the compounds' affinity at the receptor subtypes. A correlation has been found in animal studies for the ω1 (BZ1) receptors in antianxiety action, memory, and motor function, whereas the ω2 (BZ2) receptors are more involved in the sedative action. The concept has been developed and is now widely held that the side-effect profile exhibited by the benzodiazepines is a result of their lack of subtype selectivity. Consequently, it is believed that an agent with selectivity for one or a specific profile of activity at all three will result in a superior if not ideal anxiolytic drug.

Radioligand binding has become an increasingly important tool in defining the potential pharmacological activity of novel chemical entities and in providing structure-activity relationship. The ligands which can interact with benzodiazepine receptors are devided into 2 classes.

1. Classical benzodiazepine structures: e.g. diazepam, lorazepam, clonazepam, flurazepam, etc.
2. Non-benzodiazepine structures are identified in at least 9 classes of structures.
   - 2.1 Pyrazolopyridines (V): e.g. etazolate, cartazolate.
   - 2.2 Triazolopyridines: e.g. CL 218872 (VI).
   - 2.3 Adenosine analogues: e.g. EMD 28422 (VII).
   - 2.4 Dibenzocycloalkenimines: e.g. MK 801 (VIII).
   - 2.5 Pyrrolidines: e.g. zopiclone (IX).
   - 2.6 Pyrazoloquinolones: e.g. CGS 9896 (X).
   - 2.7 Quinoline derivatives: e.g. PK 8165 (XI) (Gardner et al,1987)
   - 2.8 Diaryltriazines: e.g. LY 81067 (XII).
   - 2.9 Azaspirodecanediones: e.g. buspirone (XIII).



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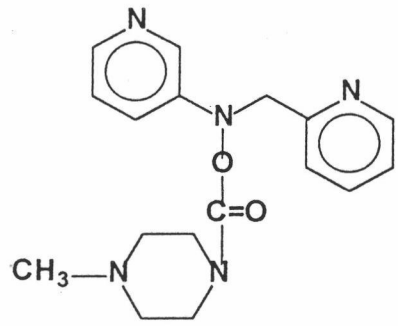

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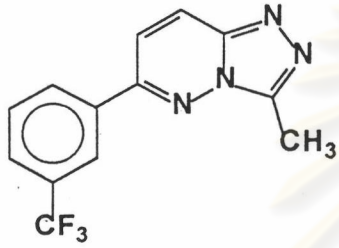

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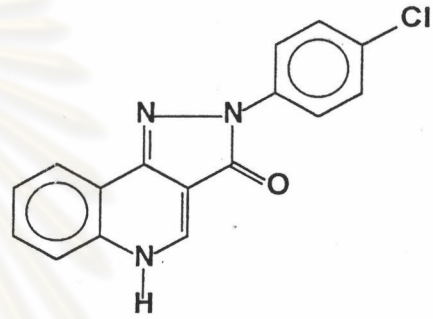

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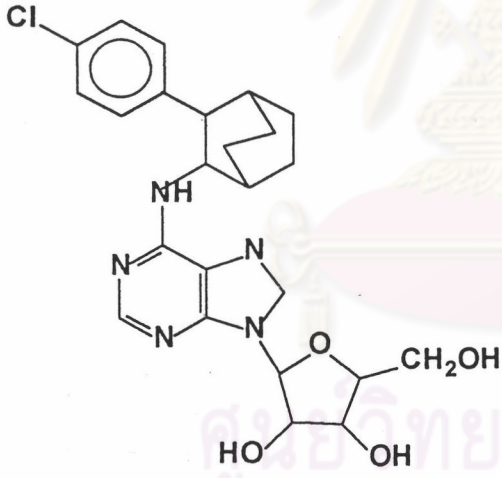

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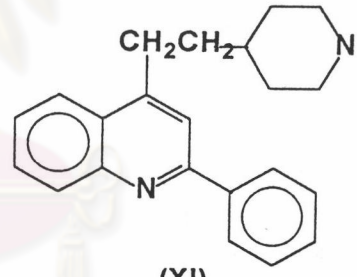

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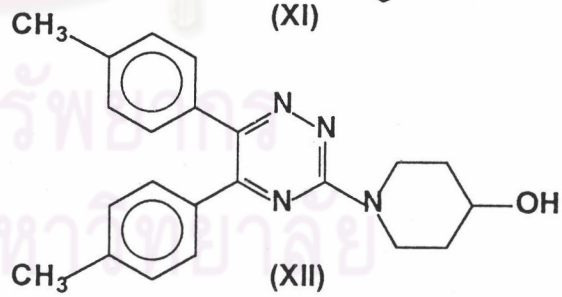

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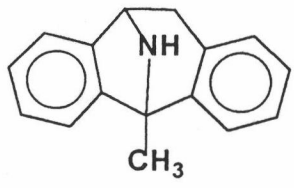

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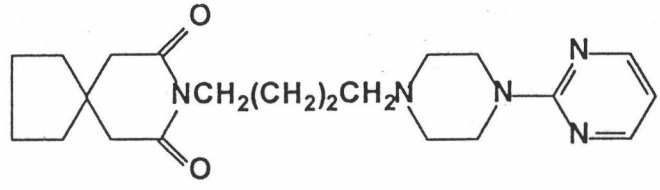
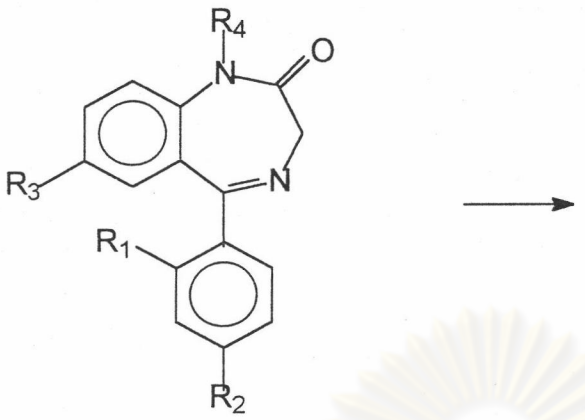


(XIII)

It has been hypothesized that there are endogeneous substances which are released physiologically to react with benzodiazepine receptors such as acetylcholine, serotonin, norepinephrine, c-AMP, dopamine, prostaglandins A1 or A2, melatonin, β-carboline, thyroxin, nicotinamide, thromboxane A2 and substance extracted from human urine called tribulin (Williams,1983). More recently, it has been suggested that benzodiazepine-like molecules exist in the brain, although this remains controversial (Gardner, 1989).

The ligands which exist for benzodiazepine receptors can be characterized into 5 groups, according to their pharmacological activity and intrinsic activity, as follow (Kenakin,1987; Gardner,1989):

1. Benzodiazepine agonists: These ligands have maximal intrinsic activity (maximal response). Examples are classical benzodiazepine derivatives (XIV), CL 218872 (VI), zopiclone (IX) and ZK 93423 (XV).



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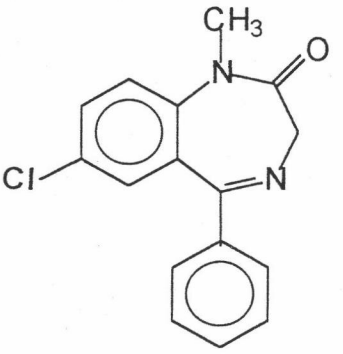
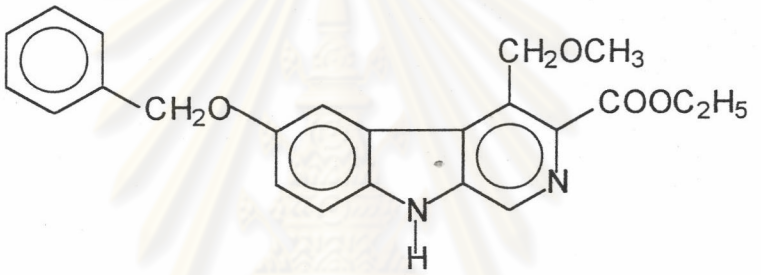


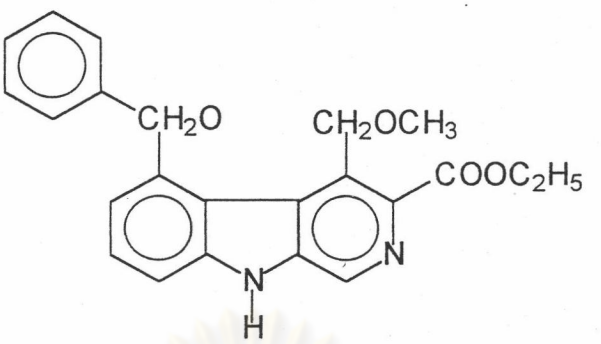
Diazepam



(XV)

Ligands in this class produce pharmacological effects such as anticonvulsant, antianxiety, ataxia or sedative according to occupancy percentage of the benzodiazepine receptors.

2. Benzodiazepine partial agonists: These ligands produce submaximal intrinsic activity, for example, CGS 9896 (X), ZK 91296 (XVI), bretazenil (XVII) (Haefely, Martin and Schoch,1990).



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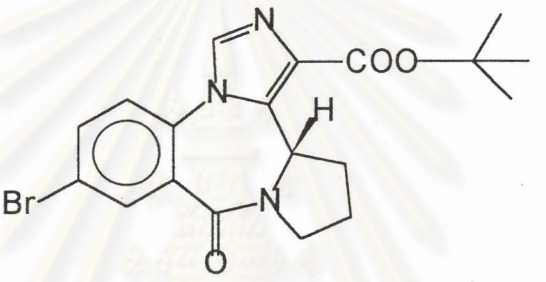
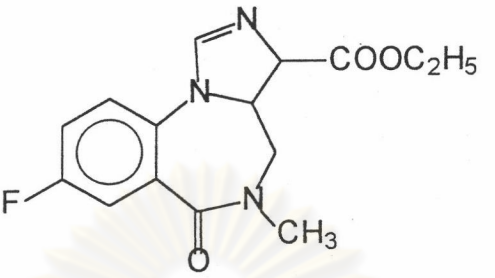


(XVII)

A partial agonist would antagonize a full agonist under the circumstances where it has occupied a large percentage of receptors but has evoked little functional effect itself (DeDeyn and Macdonald,1987). The ligands in this class are used as anticonvulsant and/or antianxiety.

3. Benzodiazepine antagonists: The pure antagonists have good affinity for the receptors, but when they occupy them, they do not produce visible response or any functional change (Skolnick et al,1982). Thus, they possess zero intrinsic activity. Benzodiazepine antagonists are able to block the actions of both agonist and inverse agonists. The example of ligands in this group are Ro 15-1788 (XVIII) and ZK 93426

(XIX). Application of these ligands is in the detoxification of benzodiazepine overdose.



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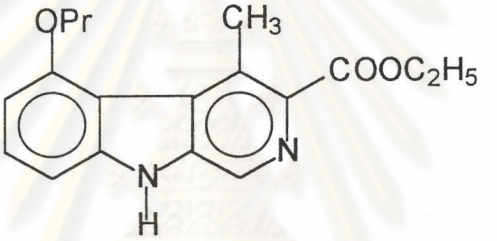
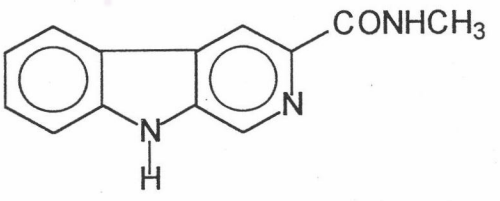


(XIX)

4. Benzodiazepine partial inverse agonist: These ligands were characterized by their submaximal negative intrinsic activity. Example are FG 7142 (XX), CGS 8216 (XXI).



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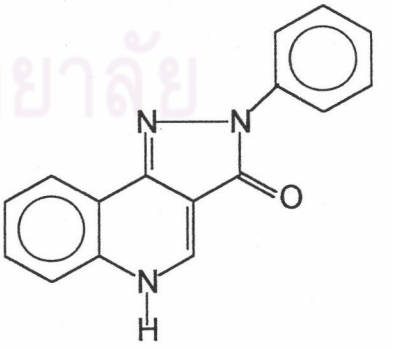
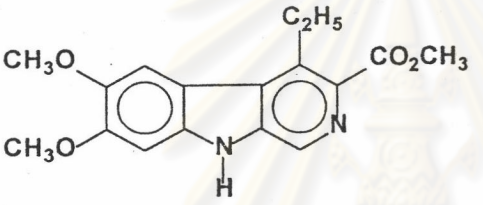


(XXI)

Presently, this group is being studied for its interesting pharmacological activities as nootropic agents (increase learning) (Brioni,1993), anoretic agent, anxiogenic or proconvulsant.

5. Benzodiazepine inverse agonists: These group of ligands produce pharmacological effects which are exactly opposite to those of the benzodiazepines (negative intrinsic activity). Example are DMCM (XXII), β-CCM (XXIII), Ro 19-4603 (XXIV).



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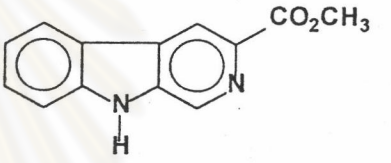

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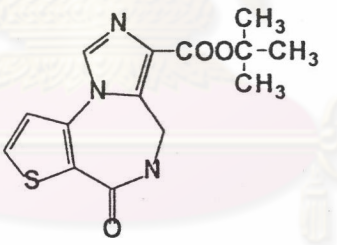


(XXIV)

These compounds produce clonic-tonic convulsion in mice and anxiety in human.

A progression of behaviors induced by full benzodiazepine agonists can be associated with increasing doses and with increasing percentage occupancy of the benzodiazepine receptors throughout the brain.

Mechanism of action of benzodiazepine ligands

Varios behavioral, electrophysiological, biochemical and pharmacological data have demonstrated that the benzodiazepines exert their therapeutic effects by binding to specific high affinity receptors in the CNS. In 1978, Tallman and colleagues observed that stimulation of GABA receptors by GABA enhanced the affinity of benzodiazepine receptors for benzodiazepines and benzodiazepines apparently facilitate GABA activity (Tallman *et al*, 1980). This raised the possibility that the two receptors were close together in neuronal membranes. The benzodiazepine receptors seem to be coupled to both the GABA receptor (especially $GABA_A$) and the chloride channel in a GABA/BZD (benzodiazepine)-receptor/chloride channel complex (Braestrup, 1982; Nogrady, 1988; DeFeudis, 1989).

The GABA receptor entity of the complex carries the recognition site for GABA, which recognizes the positive and negative charge within a certain distance in the GABA molecule. When GABA occupies its receptor, there is a conformational shift in the GABA receptor which in turn increases the affinity of benzodiazepine receptor and increases the probability that chloride channel will open, allowing chloride ions to float freely, usually from the outside into the cell (Study and Barker, 1982; Olsen and Tobin, 1990). The cell thus attains a more negative charge inside and is more difficult to excite, inhibition has occurred. Remarkably little happens to chloride channels or to GABA receptors when benzodiazepine occupy benzodiazepine receptor alones.

However, when GABA is added to a neuron on top of a benzodiazepine, it produces a greater chloride flux through the neuronal membrane than when the benzodiazepine is added alone. Some authors have suggested that ligands could be classified into 4 types based on the hypothesis of 3 binding sites involved in the benzodiazepine receptor (Fujimoto *et al*,1982). Another model has been constructed based on the action of benzodiazepine in displacing GABA-modulin, an allosteric antagonist of (Nogrady,1988).

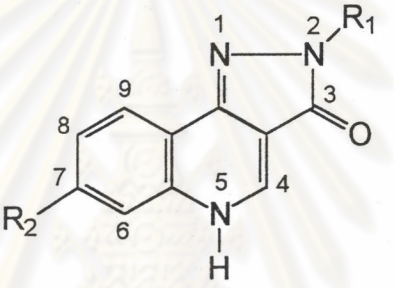
In addition to GABA, several other agents facilitate the binding of benzodiazepine agonists to central benzodiazepine receptor sites, including barbiturates, avermectin (a novel antihelmintic agent), diphenylhydantoin, glycine, etc.

The undesirable effect of benzodiazepines in anxiety disorder are its hypnotic effect and its muscle relaxant effect (ataxia). Although these ancillary pharmacological activities are well-tolerated by most individuals, we still have to design and synthesize more anxioselective compounds.

Benzodiazepine partial agonists are another choice for anxioselectivity with low toxicity. So, there are needs to design and synthesis new ligands for benzodiazepine receptors in order to solve the undesirable side effects.

Pyrazoloquinolones, a group of non-benzodiazepine derivatives, is chosen as the prototype for the synthesis of new lead compounds. SAR study of pyrazoloquinolones suggested that some essential factors for their binding to the receptors (affinity to the receptors) are: (Forbes *et al*,1990)

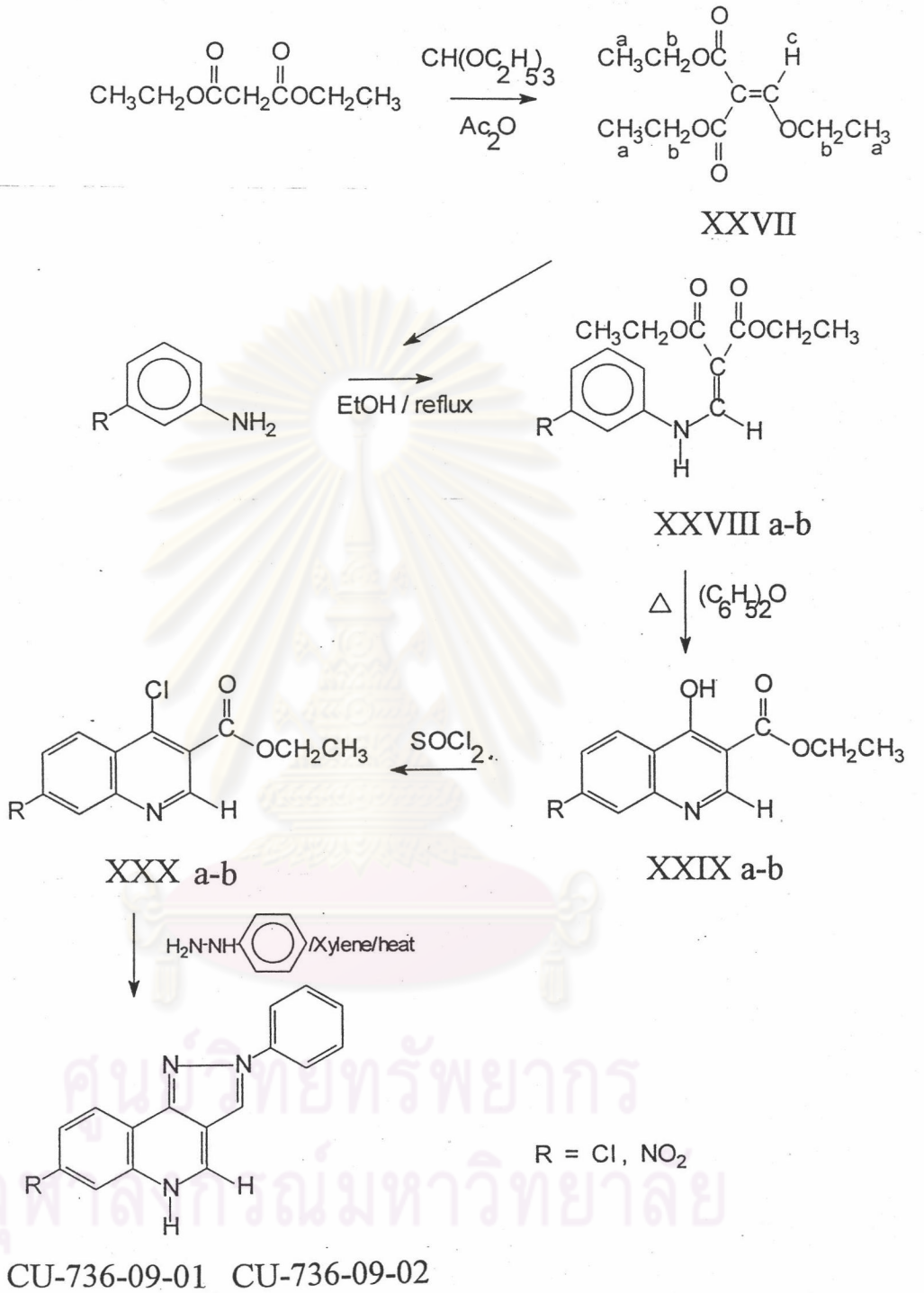
- Aromaticity of the molecule.
- Planarity of the molecule.
- Size of the molecule.



(XXV)

For this study, the systhesis of pyrazoloquinolone series substitute at position 2 with phenyl ring and at position 7 with either chloro or nitro group, is expected to give compounds with better anxioselective antianxiety activity.

The synthetic approach to the preparation of target compounds was outlined in Scheme 1.



Scheme 1 Synthesis procedure for 7-chloro and 7-nitro-pyrazolone derivatives