Chapter 1

Introduction

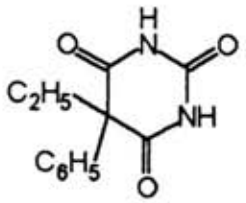
The term anxiety is a complex of subjective feelings and characteristic behaviors. The subjective feelings consist of tension, apprehension, fear, worry, and difficulty with thinking or concentrating. These feelings are usually accompanied by behavioral signs and symptoms of trembling, tremors, muscle tension, restlessness, and fatigue with autonomic hyperactivity in the respiratory (overactivity of adrenegic system in the CNS), 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. For example, intense chronically sustained anxiety or unrealistic worry about oneself or a close relative, such as a parent or child, can be truly 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 and Reynard, 1992)

Among the identified anxiety disorders are generalized anxiety disorder, panic disorder, agoraphobia, social phobia, simple phobia, post traumatic stress syndrome, and obsessive-compulsive disorder. Anxiety is also a common concomitant of many organic diseases such as hypoglycemia, anemia, vitamin B_{12} deficiency, hyperthyroidism, coronary heart disease, and mitral valve prolapse. Anxiety may also be a prominent symptom in patients in many of the other psychiatric diagnostic categories - those with personality disorders, mood (affective) disorders or schizophrenia. It is important to

recognize that anxiety symptoms are commonly produced or aggravated by a wide variety of drugs including, notably caffeine, theophylline, ephedrine, amphetamines, cocaine, thyroid hormones, digitalis, imipramine, indomethacin, baclofen, levodopa, propanolol, as well as rebound or withdrawal from alcohol or benzodiazepine use, endogenous substances or due to the situation (Smith and Reynard, 1992).

In the past it was usual to classify and discuss the agents for psychiatric disorders either according to their chemical class or according to their general pharmacological effects. But now drugs are classified according to their uses in the therapy of identified disease or conditions. Antianxiety drugs are the group of drug that can antagonize and relieve anxiety in many of the syndromes listed earlier. Many classes of drugs use for treatment of anxiety such as (Baldessarini, 1990):

- Barbiturate group: Phenobarbital (I).
- Propanediol carbamates: Meprobamate (II).
- Antihistamines: Hydroxyzine (III).
- β-adrenergic antagonists: Propanolol (IV).
- Benzodiazepines and non-benzodiazepines structures: To be mentioned later.



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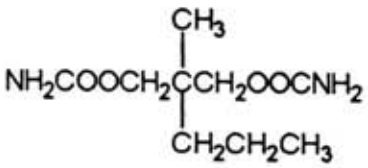

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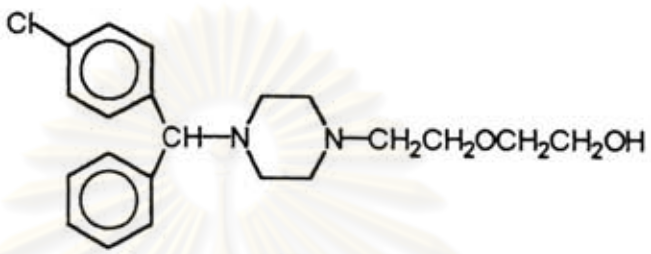

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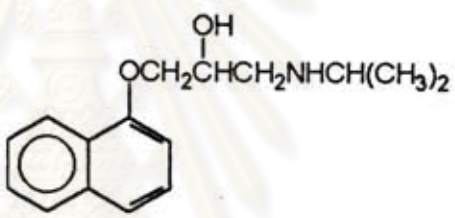


(IV)

The drug in each group have many indication. So there are some problems in using of these drugs such as: side effects, tolerance and withdrawal symtoms etc.. Since the discovery of the antianxiety and calming effects of a newly synthesized novel class of compounds, the benzodiazepines, in the late 1950's signaled the beginning of a new era of more effective and safe treatment of a variety of anxiety states.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. In recent time, benzodiazepines were found to induced memory deficits (Kulkarni and Sharma,1990 ; Brioni, 1993). Over a period about 10 years, a large number of benzodiazepine molecules were synthesized and many of them 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 radio ligand binding studies using ^{3}H-diazepam (Braestrup and Nielsen, 1982 ; Braestrup et al, 1984). Benzodiazepine receptors are localized in brain (such as on limbic system, cortical association areas, reticular or cerebellar system) and peripheral tissue (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 selectitvity for benzodiazepine, ω_1 , ω_2 and ω_3 receptor subtypes may account for the varied pharmacological action of known anxiolytics which owe their activity to this mechanism of action. This classification and widely accepted proposal has stimulated renewed interest in the discovery of novel anxiolytics in which the pharmacological profile is fined-tuned by the compounds' affinity at the receptor subtypes. The designation ω_1 , ω_2 and ω_3 receptor subtypes are replacing the use of BZ_1 , BZ_2 and BZ_p (receptor at peripheral sites) , respectively (Browne and Shaw,1991). A correlation has been found in animal studies for the ω_1 (BZ_1) receptors in antianxiety action, memory , and motor function , whereas the ω_2 (BZ_2) receptors are more involved the sedative action (Smith and Reynard,1992 ; Kalent and Roschlau, 1989). 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 noval chemical entities and in providing structure-

activity relationship. The ligands which can interact with benzodiazepine receptors are devided into 2 classes.

1. Classical benzodiazepines structure: Diazepam, Lorazepam, Clonazepam, Flurazepam etc.

2. Non-benzodiazepines structure are identified in at least 9 classes of structure (Williams, 1983).

2.1 Pyrazolopyridine (V) : Etazolate, Cartazolate (Bare et al, 1989).

2.2 Triazolopyridine : CL 218872 (VI).

2.3 Adenosine analogue : EMD 28422 (VII).

2.4 Dibenzocycloalkenimine : MK 801 (VIII).

2.5 Pyrrollopyrazine : Zopiclone (IX).

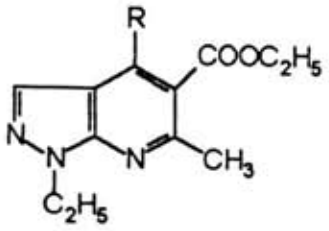
2.6 Pyrazoloquinolone : CGS 9896 (X).

2.7 Quinoline derivatives : PK 8165 (XI) (Gardner et al, 1987).

2.8 Diaryltriazine : LY 81067 (XII).

2.9 Azaspirodecanedione : Buspirone (XIII).

It has been hypothesized that there are endogeneous substances which are released physiologically to react with benzodiazepine receptors such as acetylcholine, serotonin, norepinephine, c-AMP, dopamine, prostaglandins A_1 or A_2, melatonin, β-carboline, thyroxin, nicotinamide, thromboxane A_2 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).



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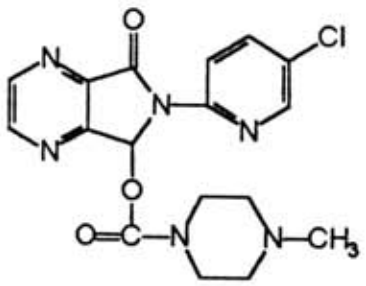

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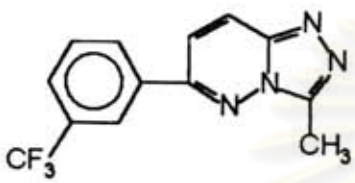

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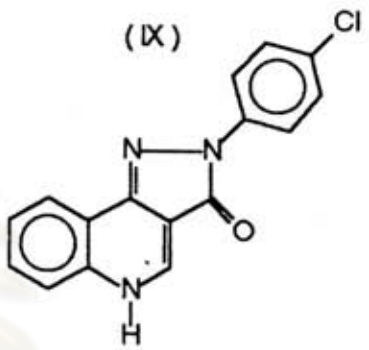

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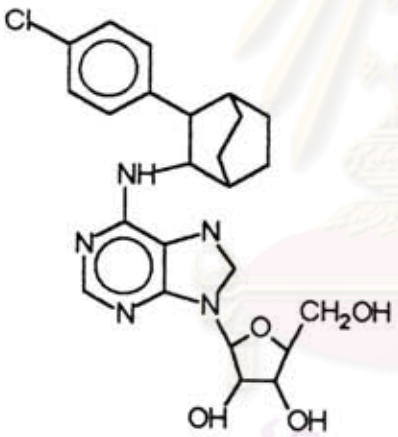

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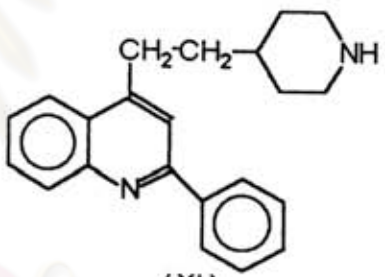

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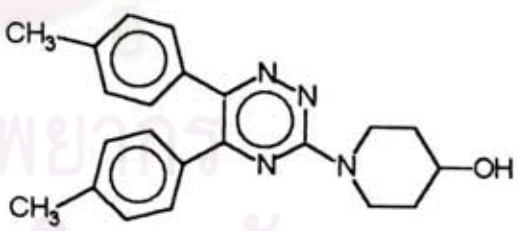

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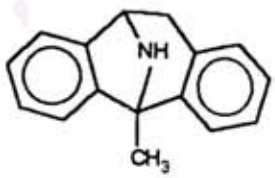

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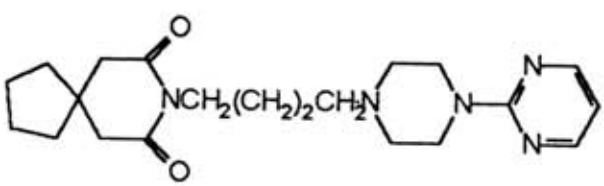
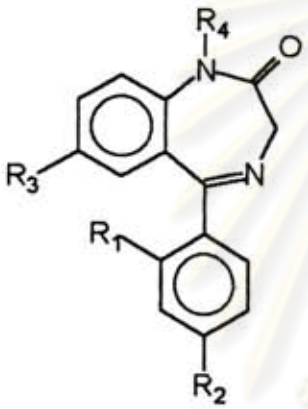


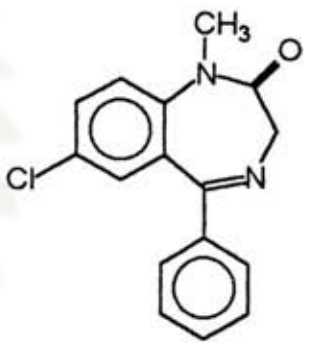
(XIII)

Figure 1. Structure of non-benzodiazepines.

The ligands which existed for benzodiazepine receptors were characterized into 5 groups according to its pharmacological activity and intrinsic activity (efficacy) (Gardner, 1989 ; Kenakin, 1987).

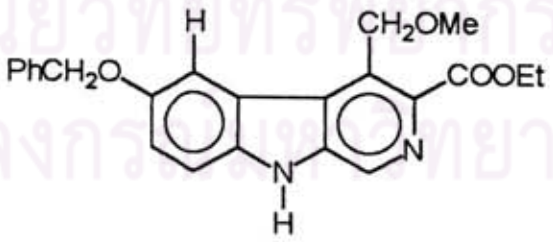
1. Benzodiazepine agonists : Ligands have maximal intrinsic activity (maximal response) such as classical benzodiazepine derivatives (XIV), CL 218872 (VI), Zopiclone (IX) and ZK 93423 (XV) etc..





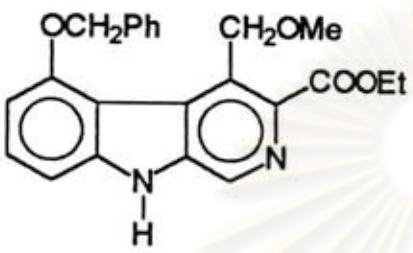
Diazepam

R_1, R_2 = H
R_3 = Cl
R_4 = CH_3

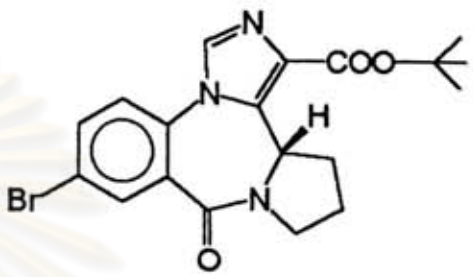


Ligands in this class have pharmacological effect as anticonvulsants, anxiolytics, ataxias, sedatives according to percentage occupancy of benzodiazepine receptors.

2. Benzodiazepine partial agonists : Ligands have submaximal intrinsic activity such as : CGS 9896 (X), ZK 91296 (XVI) and Bretazenil (XVII) etc. (Haefely, Martin and Schoch, 1990).



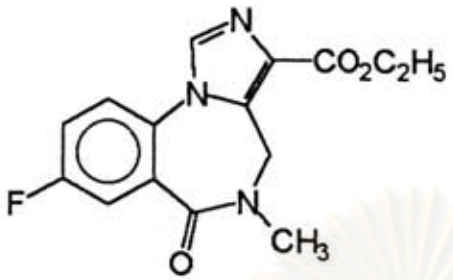
(XVI)



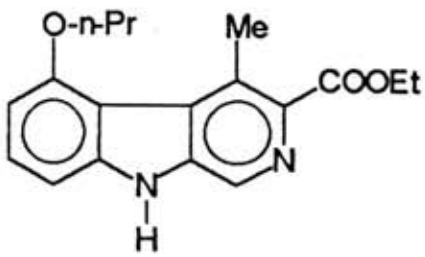
(XVII)

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

3. Benzodiazepine antagonists :The pure antagonists have good affinity for the receptors, but when they occupy the receptors, they do not produce visible response or any functional change (Skolnick, 1982).Thus they possesses zero intrinsic activity. Benzodiazepine antagonists are able to block the actions of both agonists and inverse agonists. The example of ligands in this group are Ro15-1788 (XVIII) and ZK 93426 (XIX) etc.. Application of these ligands are used for detoxify overdose of benzodiazepines.

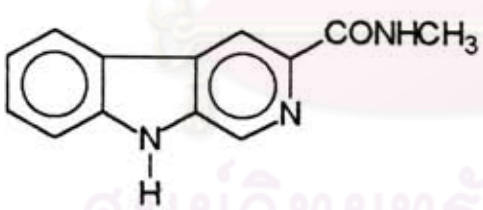


(XVIII)

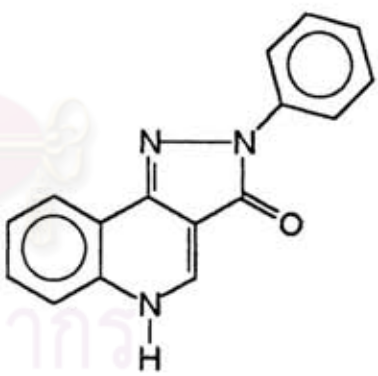


(XIX)

4. Benzodiazepine partial inverse agonists : Ligands were characterized submaximal negative intrinsic activity such as FG 7142 (XX) and CGS 8216 (XXI) etc. (In some paper CGS 8216 is classified as benzodiazepine antagonists).



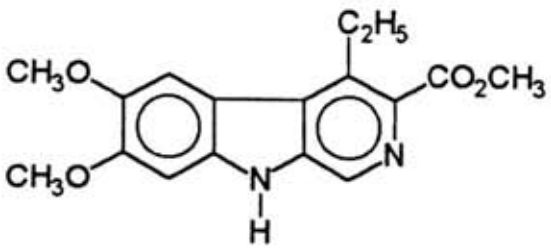
(XX)



(XXI)

This group of drug are also display pharmacological activities as nootropics (Brioni, 1993), anoretic agents, anxiogenics or proconvulsants.

5. Benzodiazepine inverse agonists : Ligands were produced pharmaclological effect which were exactly opposite to those of benzodiazepine (negative intrinsic activity) such as DMCM (XXII), β-CCM (XXIII) and Ro 19-4603 (XXIV) etc..



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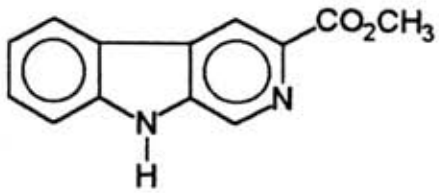

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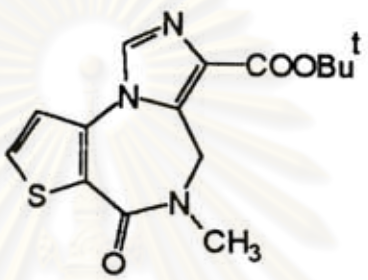


(XXIV)

Ligands in this group also produced clonic-tonic convulsion in mice and anxiety in human (Williams, 1983).

note : Intrinsic activity is the ability of a receptor ligand to induce a conformation change in the ligand receptor complex as a first step to transducing receptor activation into pharmacological effect (Figure 2.).

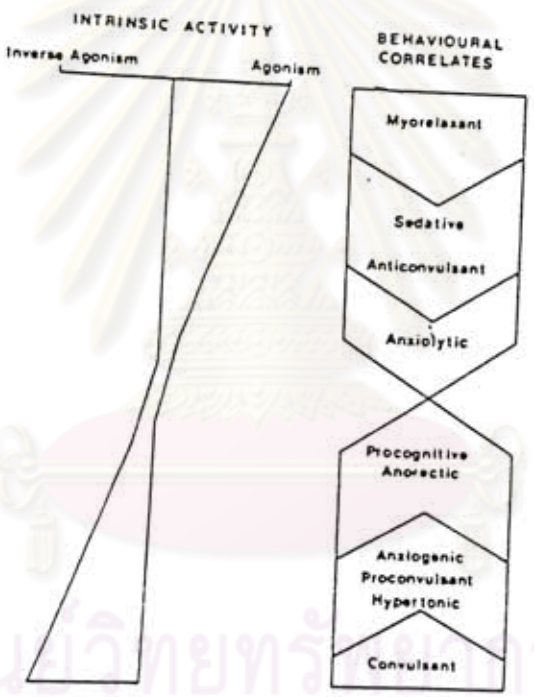


Figure 2. Schematic representation of the relationship between some clinically relevent behaviors and the degree of activation of central benzodiazepine receptors. The bidirectional nature of this continuum of behaviors is illustrated. This scheme is the basis of a working hypothesis which explains the behavioral profiles of benzodiazepine receptor ligands.

Mechanism of action of benzodiazepine ligands.

From various behavioral, electrophysiological biochemical and pharmacological data have demonstrated that the benzodiazepine exert their therapeutic effects by binding to the specific high affinity receptors in the CNS. In 1978, Tallman and colleagues observed that stimulation of GABA receptors by GABA (a major inhibitory neurotransmitters) enhanced the affinity of benzodiazepine receptorsfor benzodiazepines and benzodiazepines apparently facilitate the GABA activity (Tallman et al, 1980). This raised the possibility that the two receptors were close together in neuronal membranes. The benzodiazepine receptors (or at least most benzodiazepine receptors) seem to be coupled to both the GABA receptor (especially $GABA_{A)}$ and the chloride channel in a GABA/BZD-receptor/chloride channel complex show in Figure 3. (Braestrup, 1982 ; Norgady, 1988 ; DeFeudis, 1989).

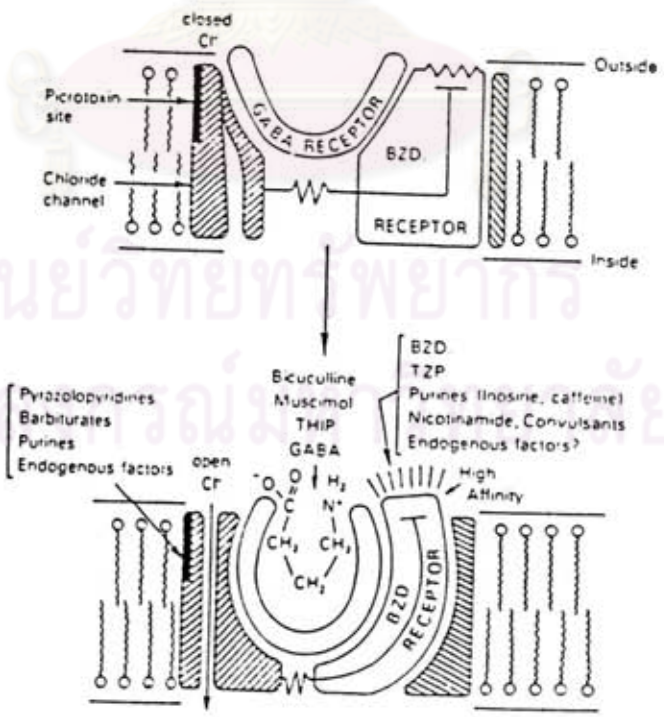


Figure 3. Model of the GABA and benzodiazepine receptor complex ; top is the close state and bottom is the open state.

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 GABA receptors (Olsen and Tobin, 1990), there is a conformational shift in the GABA receptor which in turn increase the affinity of benzodiazepine receptor and increase the probability that chloride channel will open, chloride ions float freely downstream normally from the outside into the cell (Study and Barker, 1982). The benzodiazepines alone rarely cause chloride channel opening (Braestrup, Schmiechen et al, 1982). The cell attains a more negative electric inside and is more difficult to excite, inhibition has occured. Remarkably little happens to chloride channels or to GABA receptors when bezodiazepine occupy benzodiazepine receptor alones, when GABA is added to a neuron ontop of a benzodiazepine it produces a greater chloride flux through the neuronal membrane than before the benzodiazepine was added alones. Some paper suggested that ligands could be classified into 4 types base on the assumption of 3 binding site in the benzodiazepine receptor (Fujimoto et al, 1982). Another model of mechanism has been constructed as the action of benzodiazepine is apparented based on their displacement of GABA-modulin. (GABA-modulin is allosteric antagonist of $GABA_A$ receptors that can competitively by the benzodiazepine.) After that it all express much same idea as the first model. (Nogrady, 1988)

In addition to GABA, several other agents facilitate the binding of benzodiazepine agonists to central benzodiazepine receptor sites, include barbiturates, avermectin (novel antihelmintic agents), diphenylhydantoin, glycine etc (Williams, 1983).

Epilepsy is a collective designature for a group of chronic central nervous system disorders having in common the repeat occurence of sudden and transitory episodes (seizers) of abnormal phenomena of motor, sensory, autonomic or psychic origin. The seizers are nearly always correlated with abnormal and excessive discharges in the brain (Rall and Schleifer, 1993). Epilepsy afflicts at least 1% of people worldwide. Epileptic

seizures are classified into many types according to theirs characteristics . The antiepileptic agents are classified into 4 groups according to chemical structure (Mereier, 1973).

1. Ureides

- Barbiturates and their structural analogs : Phenobarbital (XXV_a), Mephobarbital (XXV_b) .

- Hydantoins and its analogs : Phenyltoin (XXVI) .

- Acylureas : Phenacemide (XXVII) .

2. Derivatives of oxazole and its structural analogs.

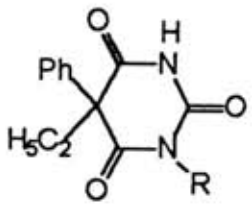
- Oxazolidinediones : Trimethadione (XXVIII) .

- Succinimides : Ethosuximide (XXIX) .

3. Derivatives of benzodiazepine and dibenzoazepine : Nitrazepam (XXX), Carbamazepine (XXXI).

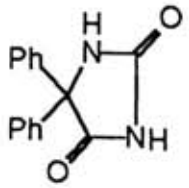
4. Various chemical compounds : Vaproic acid (XXXII), floresone (XXXIII) etc.

Benzodiazepines prevent the pentylenetetrazol-induced seizures, it is more prominent than their modification of the maximal electroshock seizure pattern. In experimental models of epilepsy, benzodiazepines suppress the spread of seizure activity produced by epileptogenic foci in the cortex, thalamus, and limbic structures but do not abolish the abnormal discharge of the focus. Further both diazepam and clonazepam suppress stimulus-induced generalized convulsions in kindled rats, but they produce little or no reduction in stimulus-induced after discharges. In agreement with these observations in animals, clonazepam has anticonvulsant activity in patients with a wide variety of seizure disorders, with the notable exception of generalized tonic-clonic seizures (Rall and Schleifer, 1993).



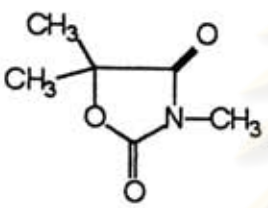
(XXV)

a) R = H
b) R = CH_3



(XXVI)

(XXVII)



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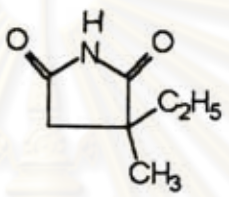

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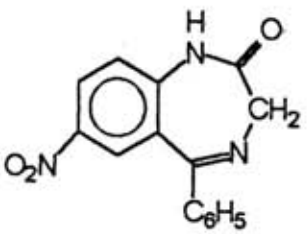

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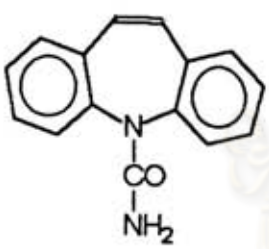

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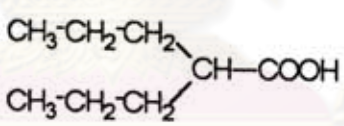
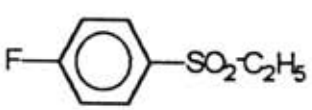


(XXXII)



(XXXIII)

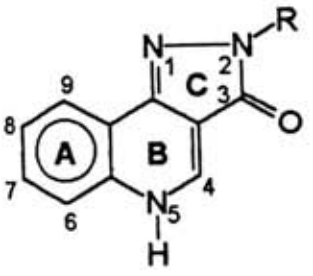
Figure 4. Chemical structure of some antiepileptic agents.

The undesirable effect of benzodiazepines in anxiety disorder are its hypnotic effect and its muscle relaxant effect (ataxia). Althrough these ancillary pharmacological activity are well tolerated by most individuals, the more anxioselective compounds are thus worth to be designed and synthesized. Some benefit of development of benzodiazepines may be produced the compounds that effect to the epileptic disorders. Unfortunately, the drugs used currently not only fail to control seizure activity in some patients, but they frequently cause side effects that range in severity from impairment of the CNS to death from aplastic anemia or hepatic failure.

Benzodiazepines partial agonists are another choice of anxioselective and specific anticonvulsant with low toxicity. So that it needs to design and synthesis new ligands for benzodiazepine receptors to solve its undesirable effects. The propose of the existence of benzodiazepine receptors is not clearly understood. While search for the yet elusive endogenous ligands continues, another synthetic compounds with diverse structure have been found to possess high affinity for benzodiazepine receptor and some of them were reported to antagonistic toward the physiological effects of benzodiazepine anxiolytics. It is important to study the physiological properties and structural requirements of various benzodiazepine-receptor binders (agonist, antagonist and inverse agonist) so that the true function of these receptors may be fully understood.

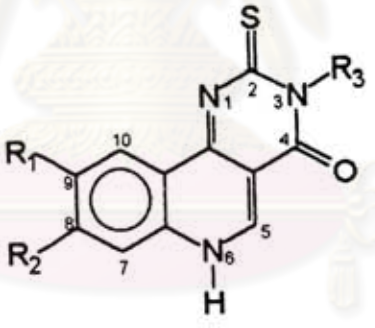
Pyrazoloquinolines (or Pyrazoloquinolinones) a non-benzodiazepine derivative which is chosen as a prototype to synthesized the new leads. The SAR studies of pyrazoloquinoline (XXXIV) suggested that some essential factors for binding to the receptors (affinity to the receptors) are: (Forbes et al, 1990)

- Aromaticity of molecule.
- Planarity of molecule (Trudell et al, 1987).
- Size of molecule.



(XXXIV)

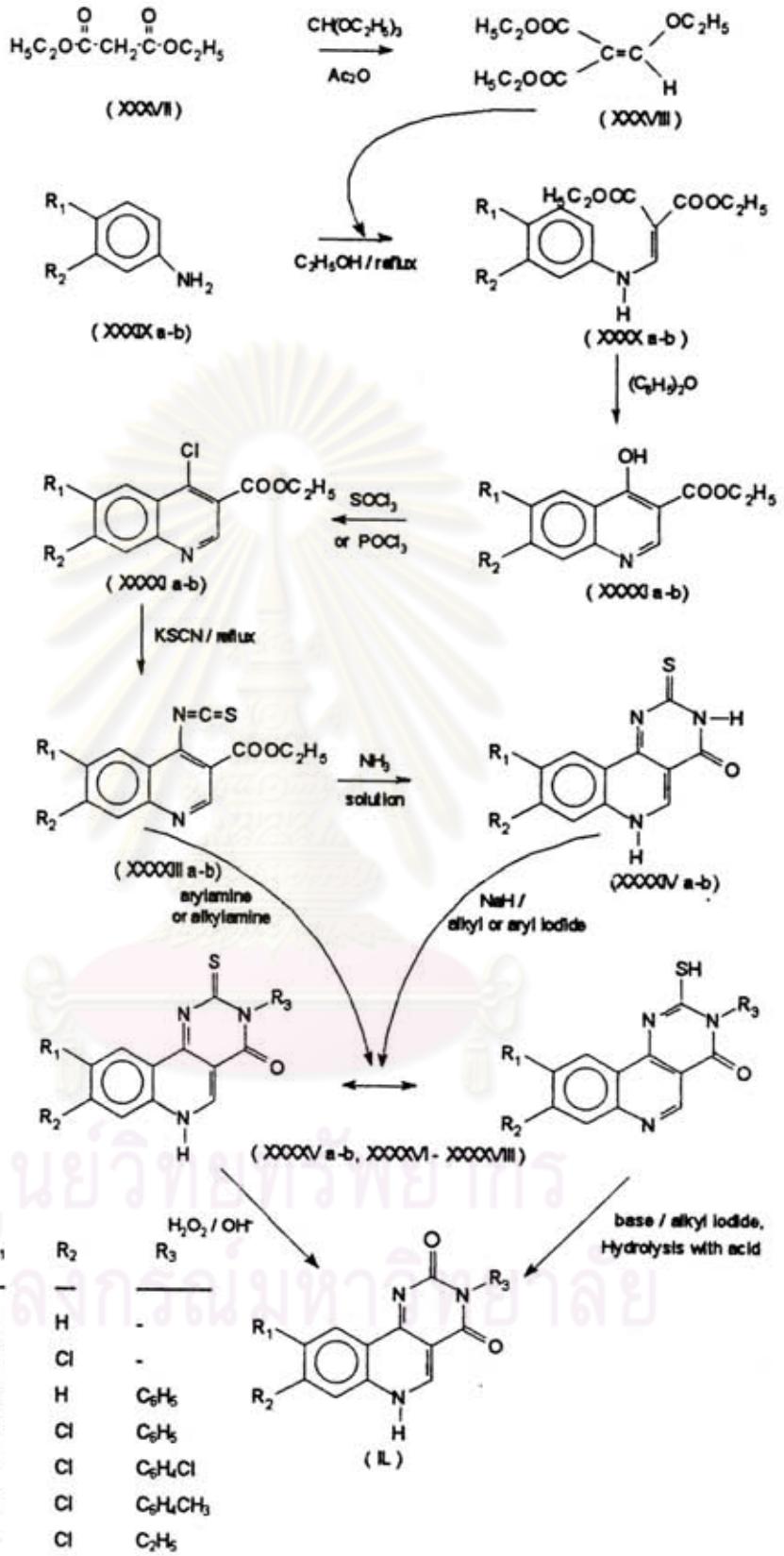
Since modification of ring C in pyrazoquinoline has not been explored, therefore changing the pyrazoline ring to thiooxopyrimidine ring may increase the aromaticity and still reserve the molecule planarity. In addition, the new lead, 2-Thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXV), has some part of molecule resemble the barbiturates, which is expected to increase affinity to benzodiazepine receptors by itself.



(XXXV)

The substitution of pyrazoloquinoline series at position 2 of (XXXIV) reflected the changing of pharmaceutical profile drastically. The aim of this experiment is the substitution of 2-Thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXV) at position 3 with alkyl group and aryl group and also varies the substituents at position 8,9 with halogen to increase the lipophilicity of the molecule. The thiooxopyrimidyl oxidation to 2,4-pyrimidine-diones derivative is also included.

The synthesis approaches for preparation of target compounds was outlined in Scheme I.



	R_1	R_2	R_3
XXXIX-XXXXIV a	H	H	-
XXXIX-XXXXIV b	F	Cl	-
XXXXV a	H	H	C_6H_5
XXXXV b, IL	F	Cl	C_6H_5
XXXXVI	F	Cl	C_6H_4Cl
XXXXVII	F	Cl	$C_6H_4CH_3$
XXXXVIII	F	Cl	C_2H_5

Scheme 1. Synthesis procedure of 2-Thiooxopyrimido-[4,3-d]-quinoline-4one derivatives and Pyrimido-[4,3-d]-quinoline-2,4-diones.