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(54) **Title:** PYRIMIDINE COMPOUNDS AS ANTI-ICTOGENIC AND/OR ANTI-EPILEPTOGENIC AGENTS

(57) **Abstract:** This invention pertains to methods and agents useful for the treatment, prevention, or inhibition of seizure-associated states, for example, the treatment, prevention, or inhibition of epileptogenesis and/or ictogenesis; and to methods for preparing anti-ictogenic and/or anti-epileptogenic agents of the invention. The invention further pertains to pharmaceutical compositions for treatment, prevention, or inhibition of seizure-associated states, as well as to kits including the anti-ictogenic and/or antiepileptogenic compounds of the invention.

PYRIMIDINE COMPOUNDS AS ANTI-ICTOGENIC AND/OR ANTI-EPILEPTOGENIC AGENTS

RELATED APPLICATIONS

This application claims priority to U.S. provisional patent application no. 60/618,296, filed October 12, 2004, identified by Attorney Docket No. NCI-189-1, entitled "Pyrimidine Compounds as Anti-Ictogenic and/or Anti-Epileptogenic Agents;" and U.S. provisional patent application no. 60/685,688, filed May 26, 2005, identified by Attorney Docket No. NCI-189-2, entitled "Pyrimidine Compounds as Anti-Ictogenic and/or Anti-Epileptogenic Agents." The entire contents of each of the foregoing patent applications are expressly incorporated by reference in their entirety including, without limitation, the specification, claims, and abstract, as well as any figures, tables, or drawings thereof.

BACKGROUND OF THE INVENTION

Epilepsy is a serious neurological condition, associated with seizures, that affects hundreds of thousands of people worldwide. Clinically, a seizure results from a sudden electrical discharge from a collection of neurons in the brain. The resulting nerve cell activity is manifested by symptoms such as uncontrollable movements.

In particular, a seizure is a single discrete clinical event caused by an excessive electrical discharge from a collection of neurons through a process termed "ictogenesis." As such, a seizure is merely the symptom of epilepsy. In fact, epilepsy is a dynamic and often progressive process, characterized by an underlying sequence of pathological transformations whereby normal brain is altered, becoming susceptible to recurrent seizures through a process termed "epileptogenesis." While it is believed that ictogenesis and epileptogenesis have certain biochemical pathways in common, the two processes are not identical. Ictogenesis (which may be described as the initiation and propagation of a seizure in time and space) is a rapid and definitive electrical/chemical event occurring over seconds or minutes. In contrast, epileptogenesis (which may be

explained as the gradual process whereby normal brain is transformed into a state susceptible to spontaneous, episodic, time-limited, recurrent seizures, through the initiation and maturation of an "epileptogenic focus") is a slow biochemical and/or histological process which generally occurs over months to years.

Epileptogenesis is a two phase process. Phase One epileptogenesis, also known as primary epileptogenesis, characterizes the initiation of the epileptogenic process prior to the first seizure, and is often the result of stroke, disease (*e.g.*, meningitis), or trauma, such as an accidental blow to the head or a surgical procedure performed on the brain. Phase Two epileptogenesis, also known as secondary epileptogenesis, refers to the process during which an individual already susceptible to seizures, becomes still more susceptible to seizures of increasing frequency and/or severity. Moreover, while the processes involved in epileptogenesis have not been definitively identified, some researchers believe that upregulation of excitatory coupling between neurons is involved, and is mediated by N-methyl-D-aspartate (NMDA) receptors. Other researchers implicate downregulation of inhibitory coupling between neurons, mediated by gamma-amino-butyric acid (GABA) receptors.

Although epileptic seizures are rarely fatal, large numbers of patients require medication to avoid the disruptive, and potentially dangerous, consequences of seizures. In many cases, medication is required for extended periods of time, and in some cases, a patient must continue to take prescription drugs for life. Furthermore, such drugs used for the management of epilepsy have side effects associated with prolonged usage, and the cost of the drugs can be considerable.

Currently, a variety of drugs are available for the management of epileptic seizures, including older anticonvulsant agents such as phenytoin, valproate and carbamazepine (ion channel blockers), as well as newer agents like felbamate, gabapentin, and tiagabine. However, the available accepted drugs for epilepsy are anticonvulsant agents, wherein the term "anticonvulsant" is synonymous with "anti-seizure" or "anti-ictogenic." While these drugs can suppress seizures by blocking ictogenesis, it is believed that they do not influence the development of epilepsy because they do not block epileptogenesis. Thus, despite the numerous drugs available for the treatment of epilepsy (*i.e.*, through suppression of the convulsions associated with epileptic seizures), there are no generally accepted drugs for the treatment of the

pathological changes that characterize epileptogenesis. Moreover, there is no generally accepted method of inhibiting the epileptogenic process, nor are there any generally accepted drugs recognized as anti-epileptogenic.

In addition to the use of anticonvulsant drugs in the management of epilepsy, focal resection surgery and vagus nerve stimulation (VNS) are also approved techniques for curing or controlling refractory seizures. Traditionally regarded as a treatment of last resort, recent progress in science and technology are making surgery safer and more effective to treat epilepsy. However, very few patients are eligible for focal resection surgery because the surgical removal of the seizure focus may result in the impairment of certain brain functions depending on its location. Additionally, concerns with respect to these treatments also relate to the expense (about US $10,000 as initial costs), as well as their invasiveness [86]. VNS is a relatively new technique based on the electrical stimulation (from a computer controlled signal generator implant) of the left vagus nerve in the neck of the patient to abort a seizure or lessen its duration.

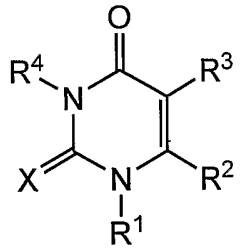
SUMMARY OF THE INVENTION

Despite the many treatment strategies currently available, about one third of the epilepsy patient population still suffers from uncontrollable seizures and drug side-effects that range from merely cosmetic to life threatening. Indeed, existing drugs do not address the underlying causes of the disorder as their therapeutic effect is limited to symptoms *i.e.*, seizures. About 30% of the epilepsy patient population does not have its seizures under control due to the lack of efficacy or unacceptable side effects of current treatments. There is, therefore, a need for drugs that would not only reduce or eliminate these seizures, but also treat, prevent, or inhibit the underlying disease.

Accordingly, the present invention is directed to the development of uracil-based compounds which may be used to treat, prevent, or inhibit seizure-associated states, *e.g.*, ranging from discrete seizure episodes to dynamic pathological transformations leading to susceptibility to recurrent seizures to epilepsy. Such compounds are capable of treating, preventing, or inhibiting the seizure-associated state directly (*e.g.*, as an anti-ictogenic agent or an anti-epileptogenic agent), or converting *in vivo* to an active agent, or acting through a combination thereof. The present invention is also directed to new

synthetic methods, for example, methods allowing production of 1-alkyl-5-bromouracil and thiouracil analogs, as well as 5,5'-biuracil analogs.

In one aspect, the invention is directed to a compound, *e.g.*, an anti-ictogenic or anti-epileptogenic compound, of the formula



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or pharmaceutically acceptable salts or esters thereof.

In another aspect, the invention pertains to a pharmaceutical composition comprising a sufficient amount of a compound of the present invention to treat, prevent, or inhibit a seizure-associated state , *e.g.*, epilepsy, in a subject in need thereof, and a pharmaceutically acceptable carrier. In a particular aspect, the pharmaceutical composition comprises a compound that is anti-ictogenic and/or anti-epileptogenic.

In another aspect, the invention relates to a method of treating, preventing, or inhibiting a seizure-associated state in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of the present invention, such that the seizure-associated state is treated, prevented, or inhibited.

An additional aspect of the invention is a method of treating, preventing, or inhibiting ictogenesis in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of the present invention, such that ictogenesis is treated, prevented, or inhibited.

Yet another aspect of the invention relates to a method of treating, preventing, or inhibiting epileptogenesis in a subject comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated or prevented.

In another aspect, the invention is directed to a kit for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that the seizure-associated state is treated, prevented, or inhibited.

In another aspect, the invention is directed to a packaged pharmaceutical composition for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting the seizure-associated state in a subject.

In yet another aspect, the invention is directed to a kit for treating, preventing, or inhibiting ictogenesis in a subject comprising a compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that ictogenesis is treated, prevented, or inhibited.

Another aspect of the invention is a packaged pharmaceutical composition for treating, preventing, or inhibiting ictogenesis in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting ictogenesis in a subject.

In another aspect, the invention pertains to a kit for treating, preventing, or inhibiting epileptogenesis in a subject comprising a compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that epileptogenesis is treated, prevented, or inhibited.

In an additional aspect, the invention is directed to a packaged pharmaceutical composition for treating, preventing, or inhibiting epileptogenesis in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting epileptogenesis in a subject.

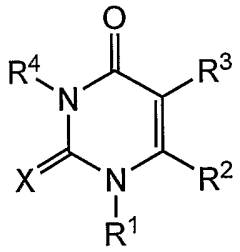
In yet another aspect, the invention is a method of treating, preventing, or inhibiting seizures, *e.g.*, seizures caused by epilepsy, in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of the present invention, such that seizures are treated, prevented, or inhibited.

In yet another aspect, the invention is a method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated, prevented, or inhibited.

Another aspect of the invention pertains to a method of treating, preventing, or inhibiting seizures in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of the present invention, such that seizures are treated, prevented, or inhibited.

Another aspect of the invention pertains to a method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated, prevented, or inhibited.

Another aspect of the invention pertains to a compound of the formula



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

R^2 is selected from the group consisting of H, CH_3,

$-CH_2CH_2$-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and $-CH_2CH(OH)$-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, $-C{\equiv}CC_4H_9$, $-CH_2N(H)$-phenyl, $-CH_2N(H)CH_2$-phenyl, $-CH_2$-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof.

In yet another aspect, the invention is directed to a method of preparing a compound of present invention, as described herein, *e.g.*, in the Examples..

DETAILED DESCRIPTION OF THE INVENTION

This invention pertains to methods and agents useful for the treatment, prevention, or inhibition of seizure-associated states; for treatment, prevention, or inhibition of epileptogenesis and/or for treatment, prevention, or inhibition of ictogenesis; and to methods for preparing compounds that are useful for the treatment, prevention, or inhibition of seizure-associated states, *e.g.*, anti-ictogenic and/or anti-epileptogenic agents. The invention further pertains to pharmaceutical compositions for treatment, prevention, or inhibition of seizure-associated states, as well as to kits including the compounds of the invention.

I. Definitions

For convenience, certain terms used in the specification, examples, and appended claims are collected here.

The language "a sufficient amount of a compound," is an amount of a compound effective, necessary, or sufficient to treat, prevent, or inhibit a seizure-associated state in a subject, *e.g.*, a therapeutically effective amount of a compound. The amount can vary depending on such factors as the size and weight of the subject, the type of illness, or the particular compound. For example, the choice of the compound can affect what constitutes a "sufficient amount". One of ordinary skill in the art would be able to study the relevant factors and make a determination regarding the sufficient amount of the compound without undue experimentation.

The language "anionic group" refers to a group that is negatively charged at physiological pH. Preferred anionic groups include carboxylate, sulfate, sulfonate, sulfinate, sulfamate, tetrazolyl, phosphate, phosphonate, phosphinate, or phosphorothioate or functional equivalents thereof. A particularly preferred anionic group is a carboxylate. "Functional equivalents" of anionic groups include bioisosteres, *e.g.*, bioisosteres of a carboxylate group. Bioisosteres encompass both classical bioisosteric equivalents and non-classical bioisosteric equivalents. Classical and non-classical bioisosteres are known in the art. *See, e.g.,* Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action*, Academic Press, Inc.: San Diego, CA, 1992, pp. 19-23.

The language "anticonvulsant agent" or "anti-ictogenic agent" includes agents which are capable of reducing, preventing or stopping of ictogenesis, inhibiting or slowing down of further ictogenesis in a subject with epilepsy or a seizure-associated state, and reducing ictogenesis in a subject with ongoing epilepsy or a seizure-associated state. The extent of the inhibition of ictogenesis may be a range from substantially complete elimination of ictogenesis, for example, to reduction of ictogenesis. Inhibition of ictogenesis is determined relative to an untreated subject, or relative to the treated subject prior to treatment, *e.g.*, determined by clinically measurable improvement in a biological function in a patient. In certain embodiments, ictogenesis may be inhibited by the compounds of the invention as described herein.

The term "anti-epileptogenic agent" includes agents which are capable of inhibiting epileptogenesis when administered to a subject.

The language "a process in a pathway associated with epileptogenesis" includes biochemical processes or events which take place during Phase One or Phase Two of epileptogenesis and lead to epileptogenic changes in tissue, *i.e.*, in tissues of the central nervous system (CNS), *e.g.*, the brain. Examples of processes in pathways associated with epileptogenesis are discussed in more detail, *infra*.

The language "a disorder associated with NMDA receptor activity," includes disorders of a subject where abnormal (*e.g.*, excessive) activity of NMDA receptors can be treated by antagonism of an NMDA receptor. For example, epilepsy is a disorder associated with excessive NMDA-mediated activity. Other non-limiting examples of

disorders associated with excessive NMDA-mediated activity include pain, stroke, anxiety (*e.g.*, schizophrenia), cerebral ischemia, Huntington's chorea, Parkinson's disease, motor neuron disease (*e.*g, ALS), Alzheimer's disease, dementias (*e.g.*, AIDS dementia and vascular dementia), and other disorders (in humans or animals) where excessive activity of NMDA receptors is a cause, at least in part, of the disorder. *See, e.g.,* Schoepp *et al.*, *Eur. J. Pharmacol.* 203:237-243 (1991); Leeson *et al.*, *J. Med. Chem.* 34:1243-1252 (1991); Kulagowski *et al.*, *J. Med. Chem.* 37:1402-1405 (1994); Mallamo *et al.*, *J. Med. Chem.* 37:4438-4448 (1994); and references cited therein.

The term "β-amino anionic compound" includes compounds having an amino group, such as $-NR^aR^b$, where R^a and R^b may each independently be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, or aryloxycarbonyl, or R^a and R^b, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring separated from an anionic group by a two-carbon spacer unit. Thus, for example, a β-amino anionic compound can be represented by the substructural formula $A\text{-}C\text{-}C\text{-}NR^aR^b$, where A is an anionic group. Preferred β-amino anionic compounds include β-amino acids and analogs thereof. In certain embodiments, the β-amino anionic compound is not β-alanine or taurine.

The term "container" includes any receptacle for holding the therapeutic formulation. For example, in one embodiment, the container is the packaging that contains the formulation. In other embodiments, the container is not the packaging that contains the formulation, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged formulation or unpackaged formulation and the instructions for use of the formulation. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the therapeutic formulation may be contained on the packaging containing the therapeutic formulation, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions can contain information pertaining to the compound's ability to perform its intended function, *e.g.*, treat, prevent, or inhibit ictogenesis and/or epileptogenesis.

The term "epilepsy" describes the clinical state associated with recurrent seizures whatever the etiology or causation.

The language "inhibition of epileptogenesis" includes reducing, preventing or stopping of epileptogenesis, inhibiting or slowing down of further epileptogenesis in a subject with epilepsy, and reducing or reversing epileptogenesis in a subject with ongoing epilepsy. For example, the extent of the inhibition of epileptogenesis may be a range, from substantially complete elimination of epileptogenesis for example, to reduction of epileptogenesis. Inhibition of epileptogenesis may be determined relative to an untreated subject, or relative to the treated subject prior to treatment, or, *e.g.*, determined by clinically measurable improvement in a biological function in a patient or by indication that the biological function in the patient does not worsen (*i.e.*, the patient does not develop epilepsy). In certain embodiments, epileptogenesis may be inhibited by the compounds of the invention as described herein.

The term "pharmacophore" is known in the art, and includes molecular moieties capable of exerting a selected biochemical effect, *e.g.*, inhibition of an enzyme, binding to a receptor, chelation of an ion, and the like. A selected pharmacophore can have more than one biochemical effect, *e.g.*, can be an inhibitor of one enzyme and an agonist of a second enzyme. A therapeutic agent can include one or more pharmacophores, which can have the same or different biochemical activities. The skilled practitioner will recognize that a number of pharmacophores with similar structures and/or properties (*e.g.*, biological effects) may be combined to predict or design an optimized or "average pharmacophore" structure. Such an average pharmacophore structure may provide a greater level of desired biological effect than the individual pharmacophores used to create the average structure.

The language "seizure-associated state" includes clinical states or processes which are characterized by one or more discrete seizure events or episodes caused by an excessive electrical discharge from a collection of neurons. Seizure-associated states include, for example, a range from single discrete seizure episodes, *e.g.*, occurring through ictogenesis, to dynamic pathological transformations leading to susceptibility to recurrent seizures, *e.g.*, occurring through epileptogenesis, to epilepsy. The language "seizure-associated state" is also intended to include states prior to clinical diagnosis of seizures or epilepsy, in subjects who are at risk to develop seizures or epilepsy, for example, who have suffered from head trauma or a traumatic brain injury (TBI). It should also be noted that the clinical state of a subject in which seizures are induced,

e.g., as the result of the administration of a convulsive agent to the subject, is also considered to be a seizure-associated state. In certain embodiments, the seizure-associated state is ictogenesis, epileptogenesis, or epilepsy.

The term "subject" includes living organisms in which a seizure associated-state, *e.g.*, epilepsy, can occur, or which are at risk for having a seizure, *e.g.*, partial seizures, generalized seizures, unclassified epileptic seizures. Examples of subjects include animals, such as mammals, including but not limited to cats, dogs, horses, pigs, cows, goats, sheep, rodents (*e.g.*, mice or rats), rabbits, squirrels, bears, and primates (*e.g.*, chimpanzees, monkeys, gorillas, apes, and humans); as well as chickens, ducks, and geese; and transgenic species thereof. In certain embodiments, the subject is human or dog. In a particular embodiment, the subject is human.

As used herein, "treatment" of a subject includes the application or administration of a composition of the invention to a subject, or application or administration of a composition of the invention to a cell or tissue from a subject that suffers from a seizure-associated state, or has a symptom of such a disease or condition, or is at risk of having (or susceptible to) a seizure or developing epilepsy, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indicia of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a subject's physical or mental well-being; or, in some situations, preventing the onset of particular conditions. The treatment or amelioration of symptoms can be based on objective or subjective parameters, including the results of a physical examination, a psychiatric evaluation, or a cognition test such as Clinical Dementia Rating (CDR) scale, Mini-mental State Examination (MMSE), Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), or another test known in the art.

In certain embodiments of the invention, the subject is in need of treatment by the methods of the invention, and is selected for treatment based on this need. A subject in need of treatment is art-recognized, and includes subjects that have been identified as having a seizure-associated state, having a symptom of a seizure-associated state, or at risk of a seizure-associated state, *e.g.*, subjects that have been exposed to a head trauma or tramatic brain injury (TBI) and would be expected, based on diagnosis, *e.g.*, medical diagnosis, to benefit from treatment (*e.g.*, curing, healing, preventing, alleviating, relieving, altering, remedying, ameliorating, improving, or affecting the seizure-associated state, the symptom of the seizure-associated state, or the risk of the seizure-associated state).

Unless specifically indicated, the chemical groups of the present invention may be substituted or unsubstituted. Further, unless specifically indicated, the chemical substituents may in turn be substituted or unsubstituted. In addition, multiple substituents may be present on a chemical group or substituent. Examples of substituents include alkyl, alkenyl, alkynyl, aryl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxyl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, formyl, trimethylsilyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amido, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, and aromatic or heteroaromatic moieties.

The term "alkyl" refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl, heterocyclyl, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably has 20 or fewer carbon atoms in the backbone. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, alkyl (*e.g.*, methyl, ethyl, propyl, butyl, pentyl, hexyl, etc.) include both "unsubstituted alkyl" and "substituted alkyl", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone, which allow the molecule to perform its intended function. Examples of substituents, which are not intended to be limiting, include moieties selected from straight or branched alkyl (preferably C_1-C_5), cycloalkyl (preferably C_3-C_8), alkoxy (preferably C_1-C_6), thioalkyl (preferably C_1-C_6), alkenyl (preferably C_2-C_6), alkynyl (preferably C_2-C_6), heterocyclic, carbocyclic, aryl (*e.g.*, phenyl), aryloxy (*e.g.*, phenoxy), aralkyl (*e.g.*, benzyl), aryloxyalkyl (*e.g.*, phenyloxyalkyl), arylacetamidoyl, alkylaryl, heteroaralkyl, alkylcarbonyl and arylcarbonyl or other such acyl group, heteroarylcarbonyl, or heteroaryl group, $(CR'R'')_{0-3}NR'R''$ (*e.g.*, $-NH_2$), $(CR'R'')_{0-3}CN$ (*e.g.*, -CN), $-NO_2$, halogen (*e.g.*, -F, -Cl, -Br, or -I), $(CR'R'')_{0-3}C(halogen)_3$ (*e.g.*, $-CF_3$), $(CR'R'')_{0-3}CH(halogen)_2$, $(CR'R'')_{0-3}CH_2(halogen)$, $(CR'R'')_{0-3}CONR'R''$, $(CR'R'')_{0-3}(CNH)NR'R''$, $(CR'R'')_{0-3}S(O)_{1-2}NR'R''$, $(CR'R'')_{0-3}CHO$, $(CR'R'')_{0-3}O(CR'R'')_{0-3}H$, $(CR'R'')_{0-3}S(O)_{0-3}R'$ (*e.g.*, $-SO_3H$, $-OSO_3H$), $(CR'R'')_{0-3}O(CR'R'')_{0-3}H$ (*e.g.*, $-CH_2OCH_3$ and $-OCH_3$), $(CR'R'')_{0-3}S(CR'R'')_{0-3}H$ (*e.g.*, -SH and $-SCH_3$), $(CR'R'')_{0-3}OH$ (*e.g.*, -OH), $(CR'R'')_{0-3}COR'$, $(CR'R'')_{0-3}$(substituted or unsubstituted phenyl), $(CR'R'')_{0-3}(C_3$-C_8 cycloalkyl), $(CR'R'')_{0-3}CO_2R'$ (*e.g.*, $-CO_2H$), or $(CR'R'')_{0-3}OR'$ group, or the side chain of any naturally occurring amino acid; wherein R' and R'' are each independently hydrogen, a C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, or aryl group. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl (*e.g.*, phenylmethyl (*i.e.*, benzyl)). An "alkylsulfide" moiety is alkyl substituted with a substituted sulfur group,

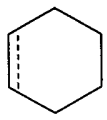
e.g., substituted with a methyl or benzyl, *i.e.*, forming a sulfide or thioether. The term "alkylsulfide" also includes alkylsulfides in different oxidation states, *e.g.*, which may further be substituted with one or more oxygens attached by single or double bonds, *e.g.*, sulfoxide or sulfone. An "alkyluracil" moiety is an alkyl substituted with a uracil group.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" includes all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds.

The term "aryl" includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles," "heteroaryls" or "heteroaromatics." The aromatic ring (*e.g.*, phenyl, indole, thiophene) can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle such as tetralin.

The terms "alkenyl" and "alkynyl" include unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively and at least two adjacent carbon atoms.

As used in the description and drawings herein, an "optional single/double bond" is represented by a solid line together with a dashed line, and refers to a covalent linkage between two carbon atoms which can be either a single bond or a double bond of either *E*- or *Z*-configuration where appropriate. For example, the structure:



can represent either cyclohexane or cyclohexene.

Unless the number of carbons is otherwise specified, "lower alkyl" means an alkyl group as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths (and at least two carbon atoms). Preferred alkyl groups are lower alkyls.

The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 4- to 7-membered rings, which ring structures include one or more heteroatoms, *e.g.*, two, three, or four. Heterocyclyl groups include pyrrolidine, oxolane, thiolane, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, including halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety.

The terms "polycyclyl" or "polycyclic group" refer to two or more cyclic rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) where two or more carbons are common to two adjoining rings, *e.g.*, the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term "aryl aldehyde," as used herein, refers to a compound represented by the formula Ar-C(O)H, where Ar is an aryl moiety (as described above) and -C(O)H is a formyl or aldehydo group. In a particular embodiment, the aryl aldehyde is a (substituted or unsubstituted) benzaldehyde. A variety of aryl aldehydes are commercially available, or can be prepared by routine procedures from commercially available precursors. Procedures for the preparation of aryl aldehydes include the Vilsmeier-Haack reaction (*See, e.g.,* Jutz, *Adv. Org. Chem.* **9**, pt. 1, 225-342 (1976)), the Gatterman reaction (Truce, *Org. React.* **9**, 37-72 (1957)), the Gatterman-Koch reaction (Crounse, *Org. React.* **5**, 290-300 (1949)), and the Reimer-Tiemann reaction (Wynberg and Meijer, *Org. React.* **28**, 1-36 (1982)).

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (*e.g.*, all enantiomers and diastereomers) are included within the scope of this invention unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis. That is, unless otherwise stipulated, any chiral carbon center may be of either (*R*)- or (*S*)-stereochemistry. Furthermore, alkenes can

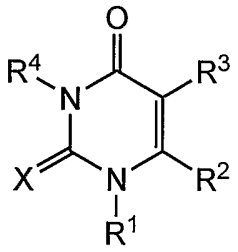
include either the *E*- or *Z*-geometry, where appropriate. Additionally, one skilled in the art will appreciate that the chemical structures as drawn may represent a number of possible tautomers, and the present invention also includes those tautomers.

II. Methods for Treating, Inhibiting, or Preventing Seizure-Associated States

In one aspect, the invention provides methods for treating, preventing, or inhibiting seizure-associated states, including epilepsy.

In another aspect, the invention provides a method for treating, preventing, or inhibiting epileptogenesis in a subject. The method includes administering to a subject in need thereof a sufficient amount of a compound of the present invention which modulates, *e.g.*, upregulates or downregulates, a process in a pathway associated with epileptogenesis such that epileptogenesis is treated, prevented, or inhibited in the subject.

Thus, in certain embodiments, compounds for administration to a subject include pyrimidines such as substituted uracils which may be used directly to treat, prevent, or inhibit seizure-associated state, or may be converted *in vivo* to active β-amino anionic compounds, or may act as a combination thereof. In one embodiment, the compound useful in the methods of the present invention can be represented by the formula



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -$CH_2CH(OH)$-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3,

$-C{\equiv}CC_4H_9$, $-CH_2N(H)$-phenyl, $-CH_2N(H)CH_2$-phenyl,
$-CH_2$-O-benzyl, an alkylsulfide, and an alkyluracil; and
R^4 is selected from the group consisting of H, benzyl, isopropyl,
p-NO_2-benzyl, and benzoyl;

or pharmaceutically acceptable salts or esters thereof. In particular embodiments of the invention, the compounds for use in accordance with the invention may be selected from compounds 1-80, discussed herein, and specifically listed in the Exemplification Section. Furthermore, many uracil analogs described herein have demonstrated broad spectrum anti-convulsant activity (active on primary or secondary seizure types), and virtually no neurotoxicity in various biological tests, outperforming established anticonvulsant drugs.

Another embodiment of the invention relates to a method of treating, preventing, or inhibiting a seizure-associated state in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of the present invention, such that the seizure-associated state is treated, prevented, or inhibited.

In another embodiment, the invention is a method of treating, preventing, or inhibiting ictogenesis in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of the present invention, such that ictogenesis is treated, prevented, or inhibited.

In an additional embodiment, the invention is a method of treating, preventing, or inhibiting epileptogenesis in a subject comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated, prevented, or inhibited.

In certain embodiments, the step of administering to the subject can include administering to the subject a compound which is metabolized to an anti-convulsant and/or anti-epileptogenic compound of the invention. For example, the methods of the invention include the use of prodrugs which are converted *in vivo* to the therapeutic compounds of the invention. *See, e.g.,* Silverman, ch. 8, cited above. Such prodrugs can be used to alter the biodistribution to allow compounds which would not typically cross the blood-brain barrier to cross the blood-brain barrier, or the pharmacokinetics of the therapeutic compound. For example, an anionic group, *e.g.*, a carboxylate group, can be esterified with an ethyl or a fatty group to yield a carboxylic ester. When the carboxylic

ester is administered to a subject, the ester can be cleaved, enzymatically or non-enzymatically, to reveal the anionic group.

In another illustrative embodiment, the methods of the invention include administering to the subject a derivative of uracil or an analog thereof (including substituted pyrimidines, uridine 5'-monophosphate (UMP) and uridine, or analogs thereof). Administration of a uracil compound or metabolite thereof can result in the *in vivo* formation of an active compound of the invention. Accordingly, in a particular embodiment, the methods of the invention may include the step of administering to a subject in need thereof a sufficient amount of a substituted or unsubstituted uracil, or a derivative or analog thereof (or a pharmaceutically acceptable salt or ester thereof), in an amount effective to treat, prevent, or inhibit a seizure-associated state , *e.g.*, by direct activity of the uracil, *in vivo* conversion of the uracil to a β-amino acid compound, or by a combination thereof. In certain embodiments, the compound of the invention is not uridine.

A. Pathways Associated with Seizure-Associated States

Normal functions of the brain rely on the fine balance between excitatory and inhibitory neuronal inputs. Glutamate (Glu) and Aspartate (Asp) are the major excitatory neurotransmitters, while GABA, Glycine [26-30] and to some extent, Taurine [31-34] are the main inhibitory neurotransmitters in the CNS. Moreover, as noted above, upregulation of excitatory coupling between neurons, mediated by N-methyl-D-aspartate (NMDA) receptors, and downregulation of inhibitory coupling between neurons, mediated by gamma-amino-butyric acid (GABA) receptors, have both been implicated in epileptogenesis. Other processes in pathways associated with epileptogenesis include release of nitric oxide (NO), a neurotransmitter implicated in epileptogenesis; release of calcium (Ca^{2+}), which may mediate damage to neurons when released in excess; neurotoxicity due to excess zinc (Zn^{2+}); neurotoxicity due to excess iron (Fe^{2+}); and neurotoxicity due to oxidative cell damage.

Accordingly, in certain embodiments, an agent to be administered to a subject to treat, prevent, or inhibit epileptogenesis may treat, prevent, or inhibit one or more processes in at least one pathway associated with epileptogenesis. For example, an agent useful for inhibition of epileptogenesis can reduce the release of, or attenuate the epileptogenic effect of, NO in brain tissue; antagonize an NMDA receptor; augment endogenous GABA inhibition; block voltage-gated ion channels; reduce the release of, reduce the free concentration of (*e.g.*, by chelation), or otherwise reduce the epileptogenic effect of cations including Ca^{2+}, Zn^{2+}, or Fe^{2+}; inhibit oxidative cell damage; or the like. In certain particular embodiments, an agent to be administered to a subject to inhibit epileptogenesis is capable of inhibiting at least two processes in at least one pathway associated with epileptogenesis.

Non-limiting examples of pharmacophores which can modulate a process in a pathway associated with epileptogenesis include:

- inhibitors of NO synthase such as L-arginine and alkylated derivatives thereof;
- antagonists of NMDA receptors such as (*R*)-α-amino acids. *See, e.g.,* Leeson, P.D. and Iverson, L.L., *J. Med. Chem.* (1994) 37:4053-4067, for a general review of inhibitors of the NMDA receptor;
- augmenters of endogenous GABA inhibition such as inactivators of GABA aminotransferase like gamma-vinyl-GABA. *See, e.g.,* Krogsgaard-Larsen, P., *et al., J. Med. Chem.* (1994) 37:2489-2505) for a review of GABA receptor agonists and antagonists;
- chelators of Ca^{2+}, Zn^{2+}, or Fe^{2+} such as EDTA, EGTA, TNTA, 2,2-bipyridine-4,4,-dicarboxylate, enterobactin, porphyrins, crown ethers, and azacrown ethers; and
- antioxidants such as vitamins C, and E; carotenoids such as β-carotene; butylated phenols, Trolox (a tocopherol analog), selenium; and glutathione.

In one particular embodiment, the agent antagonizes an NMDA receptor and augments endogenous GABA inhibition. In certain particular embodiments, the agent is administered orally. Preferably, after oral administration, the agent is transported to the nervous system of the subject by an active transport shuttle mechanism. A non-limiting example of an active transport shuttle is the large neutral amino acid transporter, which is capable of transporting amino acids across the blood-brain barrier (BBB).

Voltage-gated ion channels

Voltage-gated ion channels can play a significant role in neuronal functions. Since seizures are associated with hypersynchronous and hyperexcitable neurons, blocking or dampening the high frequency repetitive neuronal firing of action potentials at the voltage-dependent sodium ion channels could constitute an important target [50].

Many of the conventional anticonvulsant drugs, including phenytoin, carbamazepine, and valproic acid appear to suppress Na^+ currents in a voltage- and use-dependent manner. For example, phenytoin binds Na^+ channels, and upon firing, the channels open, which in turn results in better binding to the channels by phenytoin, *i.e.*, selective state-dependent binding. This means that the more the neuron is depolarized, and the more it fires, the more efficient the drug is in suppressing repetitive firings [51]. Thus, these drugs appear to be effective against the high frequency repetitive firing required for the generation and progression of an epileptic seizure without significant interference with the normal firing modes. As indicated below, such drugs are active in the MES-induced seizure test.

Anticonvulsant effect of conventional drugs in different animal models of seizures and human epilepsy. [57]

	Anticonvulsant activity in animal models of seizures			Clinical efficacy (in humans)			
	MES test	PTZ test	Amygdala-Kindling test	Partial seizures	Generalized seizures		
					Tonic-clonic	Absence	Myoclonic
Known Anticonvulsant Drugs							
Carbamazepine	+	NE	+	+	+	NE	NE
Phenytoin	+	NE	+	+	+	NE	NE
Phenobarbital	+	+	+	+	+	NE	+
Primidone	+	+	+	+	+	NE	+
Valproic acid	+	+	+	+	+	+	+
Benzodiazepines*	+	+	+	+	+	+	+
Ethosuximide	NE	+	NE	NE	NE	+	±
Lamotrigine	+	NE	+	+	+	+	+
Topiramate	+	NE	+	+	+	±	+
Oxcarbazepine	+	±	ND	+	ND	NE	NE
Felbamate	+	+	+	+	+	±	+
Vigabatrin	NE	+	+	+	ND	NE	NE
Tiagabine	NE	+	+	+	ND	ND	NE
Gabapentin	±	±	+	+	ND	NE	NE
Levetiracetam	NE	NE	+	+	±	ND	+

+: effective; ±: inconsistent; NE: not effective; ND: no data available or found
*: Reference [57] further describes collective term

Conventional anticonvulsant drugs including lamotrigine, oxcarbazepine and topiramate exert their anticonvulsant effects through the blockage of repetitive firings by inhibition of voltage-gated Na^+ channels [52-54]. In contrast, benzodiazepines and barbiturates may also block these firings but only at toxic concentrations [55].

Other voltage-gated ion channels that are targeted in antiseizure drug development include those affecting calcium and potassium ions. In fact, phenytoin, benzodiazepines, and barbiturates are known to affect K^+ currents or the Ca^{2+} dependent K^+ current. Additionally, calcium ion entry into neurons occurs through several heterogeneous types of voltage-dependent calcium ion channels composed of multiple

subunits. Moreover, there are six different types of Ca^{2+} ion channels known as the L-, T-, N-, O-, Q-, and R-types; each one having its own pharmacological properties.

Although several conventional anticonvulsant drugs reduce Ca^{2+} currents at higher (toxic) concentrations, the anticonvulsant drug ethosuximide is known to selectively reduce low threshold T-type Ca^{2+} channel currents in a voltage-dependent manner at therapeutic drug concentration [56]. This anticonvulsant is effective in absence seizure but ineffective in focal seizures or suppressing the development of primary generalized tonic-clonic seizures (as shown in the table below). However, absence seizures may also be suppressed by benzodiazepines through the enhancement of GABA receptors.

Cellular mechanisms of action of conventional anticonvulsant drugs [58-60]

Drug	Blockage of voltage-dependent Na^+ channels	Blockage of T-type Ca^{2+} ion channels	Potentiation of GABAergic mechanisms	Blockage of glutamatergic mechanisms
Carbamazepine	++	ND	±	±
Phenytoin	++	NE	±	±
Phenobarbital	+	NE	+	+
Valproic acid	++	NE	+	±
Benzodiazepines	+	NE	++	NE
Ethosuximide	NE	+	NE	NE
Lamotrigine	++	NE	NE	±
Topiramate	+	ND	+	+
Oxcarbazepine	++	ND	NE	ND
Felbamate	+	ND	+	++
Vigabatrin	ND	ND	++	ND
Tiagabine	ND	ND	++	ND
Gabapentin	±	NE	+	±

+, ++: effective; ±: inconsistent; NE: not effective in therapeutically relevant concentrations; ND: no data available or found

Enhancement of GABA-mediated inhibition

The main inhibitory neurotransmitters in the CNS are glycine and GABA with the latter being the most predominant and effective in the brain inhibition processes. Glycine is an agonist at two neurotransmitter receptor sites, one sensitive to inhibition by strychnine and linked to the inhibitory glycine-gated chloride channel and the other a co-agonist site on the excitatory amino acid NMDA cation channel complex [61, 62]. However, all neurons within the mammalian CNS, including the human brain, have GABA receptors that produce hyperpolarizing inhibition when activated. Moreover, it has been well established that reduction of GABA-mediated inhibition results in seizures while enhancement of inhibition is associated with the suppression of seizures [63].

There exists three types of GABA receptors in the CNS known as the $GABA_A$, $GABA_B$, and $GABA_C$ receptors. However, in certain embodiments, the $GABA_A$ receptor appears to be more important in controlling neuronal hyperexcitability, and thus seizure propagation. In particular, the GABA that is enzymatically synthesized from glutamate by the glutamic acid (or glutamate) decarboxylase (GAD) binds to a $GABA_A$ receptor, and mediates inhibition by opening the chloride ion channels and hyperpolarizing the membrane. After synaptic release, GABA is taken up into nerve cells or glial cells through a GABA uptake carrier. Subsequently, in the neuron, GABA is either re-released or broken down by the GABA transaminase (GABA-T) into succinic acid semialdehyde (SSA) as in glial cells.

The $GABA_A$ receptor is associated with an ion channel permeable to chloride ions, GABA increases the probability of the ion channel opening that increases the inward flow of chloride ions through the nerve cell membrane and inhibits neuronal firing. Drugs such as the barbiturates and benzodiazepines exert their anticonvulsant activity by modifying GABA receptor mediated responses *via* associated modulatory sites to potentiate the effect on chloride ion conductance [64-66].

In fact, there are several known anticonvulsant drugs that exert their effects by enhancing the actions of GABA at $GABA_A$ receptors. Barbiturates such as phenobarbital act to prolong inhibition by keeping GABA-activated channels open longer; and benzodiazepines such as diazepam, clonazepam and clobazam act to increase the frequency of chloride ion channel opening, thereby making inhibitory postsynaptic

potentials larger [67]. These two classes of drugs bind to specific sites on the GABA receptor and allosterically alter the channel properties. Other approaches to enhance GABA mediated inhibition include, but are not limited to:

- ✓ GABA mimetics, since GABA is not sufficiently lipophilic to effectively cross the blood brain-barrier when systematically administered;
- ✓ GABA-T enzyme inhibitors, preventing the breakdown of GABA; and
- ✓ GABA uptake blockers in neurons or glial cells [68].

The most direct way of enhancing inhibition is with drugs that enter the brain and are converted into either GABA (or structurally related compounds with $GABA_A$ receptor agonist activity) or analogs of other endogenous inhibitory neurotransmitters such as glycine. Successful applications of this approach, at least in animal models, have been the pro-drugs progabide, and milacemide, which readily cross the BBB and are converted *in vivo* to neurotransmitters GABA and glycine, respectively. They were found to be active in controlling seizures in several animal models but their clinical trials were halted due to insignificant efficacy in humans [69].

Inhibition enhancement may also be achieved by interfering with the GABA catabolic enzyme GABA-T. This may increase the synaptic availability of GABA as its breakdown to SSA is decreased or prevented. In principle, GABA-T inhibitors should potentiate the action of synaptically released GABA without producing generalized inhibitory effects throughout the brain, as do direct–acting GABA agonists. This approach led to the development and subsequent marketing of the anticonvulsant drug, vigabatrin, an irreversible GABA-T inhibitor [70].

The third approach for enhancing synaptic GABA levels is the blockage of GABA uptake into neurons or glia. Just like GABA-T inhibitors, GABA uptake blockers should be expected to selectively potentiate the inhibitory effects of synaptically released GABA [71]. Conventional blockers of GABA uptake, such as nipecotic acid and guavacine, have anticonvulsant activity when injected intracerebroventricularly, but they do not cross the BBB [72]. Thus it increases the extracellular concentration of GABA in the hippocampus and prolongs the inhibitory effects of synaptically released GABA.

In case of prolonged seizure activity, synaptically available GABA may be depleted, and the blockage of glial uptake may be more effective in suppressing discharges than the blockage of neuronal uptake [74]. GABA uptake is performed by carriers or transporters, which exist in four different types known as the GAT-1, GAT-2, GAT-3 and GAT-4. GAT-1, which is found in both neurons and glial cells, and is readily inhibited by nipecotic acid or its analogue Tiagabine, and exclusively transports GABA [75]. GAT-2 and GAT-3, which have a glial localization, bind both GABA and β-alanine. GAT-4 may be absent from the CNS.

Diminution of Glutamate-Mediated Excitation

Aspartate and Glutamate are the main excitatory neurotransmitters in the brain, the latter being more predominant. When presynaptically released, Glutamate produces an excitatory postsynaptic current that may be resolved into fast and slow (or long-lasting) components. The latter being mediated through NMDA receptors while the former is controlled by the non-NMDA ionotropic glutamate receptor-channels AMPA and Kainate [79, 80]. Thus, Glutamate acts postsynaptically on three classes of ion channel-linked receptors named after their preferred agonists: N-Methyl-D-aspartic Acid (NMDA), kainic acid and alpha–Amino-3-hydroxy-5-Methyl-4-isoxazole Propionic Acid (AMPA) or quisqualic acid. The latter two molecules, AMPA and quisqualic acid share the same receptor, which is usually referred to as the AMPA receptor.

Of the three receptors, the NMDA receptor has been shown to exhibit properties that facilitate the burst firing and Paroxysmal Depolarizing Shift (PDS) typical of epileptic discharges [82]. Moreover, excessive activation of NMDA receptors is thought to mediate Ca^{2+} dependent neurotoxicity associated with trauma, epilepsy and several neurodegenerative diseases.

NMDA receptors are present in virtually every neuronal cell [74]. They are widely distributed in the brain and spinal cord, with the highest densities in cerebral cortex and hippocampus, areas of the brain that seem to be the most susceptible to epilepsy pathogenesis. These receptors, when activated, control ion channel permeability to Na^{+}, K^{+}, and Ca^{2+}. However, the channel is blocked by Mg^{2+} during normal synaptic transmission and is voltage-dependent. For activation, NMDA

receptors require occupation of both the glutamate and glycine subunits. Thus, NMDA receptors can be blocked by competitive antagonists acting at either the glutamate or the glycine (co-agonist) sites.

The NMDA receptor is associated with several modulators and pharmacological antagonists which act at its different sites. These include the NMDA (or glutamate) site, the glycine (co-agonist) site, the Zn^{2+} modulatory site, the redox modulatory site, the polyamine site, the Mg^{2+}-block site, the H^{+} ion modulation sites as well as other sites involved in the binding of open-channel blockers such as memantine and the dissociative anesthetic agents phencyclidine (1-(1-PhenylCyclohexyl)Piperidine (PCP) and dizocilpine, also known as MK-801.

B. Additional Methods of the Invention

In one embodiment, the invention provides a method for treating, preventing, or inhibiting a seizure-associated state in a subject. The method includes the step of administering to a subject in need thereof a sufficient amount of an agent which a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; and/or b) has at least one activity selected from the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that seizure-associated state is treated, prevented, or inhibited in the subject.

Accordingly, in certain embodiments, an active agent of the invention antagonizes NMDA receptors, *e.g.*, by binding to the glycine binding site of the NMDA receptors. In certain embodiments, the agent augments GABA inhibition by decreasing glial GABA uptake. In certain other embodiments, the agent is administered orally. In yet other embodiments, the method further includes administering the agent in a pharmaceutically acceptable vehicle.

In another embodiment, the invention provides a method for treating, preventing, or inhibiting both ictogenesis and epileptogenesis in a subject, *i.e.*, concurrently or sequentially. The method includes the step of administering to a subject in need thereof a sufficient amount of an agent which a) blocks sodium or calcium ion channels, or opens potassium or chloride ion channels; and b) has at least one activity selected from

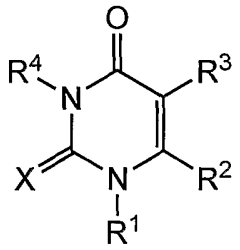
the group consisting of NMDA receptor antagonism; augmentation of endogenous GABA inhibition; calcium binding; iron binding; zinc binding; NO synthase inhibition; and antioxidant activity; such that epileptogenesis is treated, prevented, or inhibited in the subject.

Moreover, it should be understood that one or more moieties or pharmacophores that are associated with the modulation the pathways described herein may be coadministered with the compounds of the invention, either as substituents of (*i.e.*, covalently linked to) the compounds of the invention or in combination treatments as separate agents. Moerover, it is within the scope the present invention that one or more existing drugs may be used in conjunction with the compounds of the present invention to achieve a therapeutic effect, wherein the existing drug may be a substituent of the compounds of the present invention or an additional agent coadministered with the compound of the invention.

For example, blockers of sodium and/or calcium ion channel activity are well known in the art and can be used as a substituent of the compounds in the methods of the present invention. Similarly, any compound which opens potassium or chloride ion channels can be used as a substituent. Antagonists of NMDA receptors and augmenters of endogenous GABA inhibition are also known to one of skill in the art and can be used used as a substituent. In particular, 2,3-quinoxalinediones are reported to have NMDA receptor antagonistic activity (*See*, *e.g.*, U.S. Patent No. 5,721,234.) Exemplary calcium and zinc chelators include moieties known in the art for chelation of divalent cations, including ethylenediaminetetraacetic acid (EDTA), ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid, and the like, in addition to those mentioned *supra*. Exemplary iron chelators include enterobactin, pyridoxal isonicotinyl hydrazones, N,N'-bis(2-hydroxybenzoyl)-ethylenediamine-N,N'-diacetic acid (HBED), and 1-substituted-2-alkyl-3-hydroxy-4-pyridones, including 1-(2'-carboxyethyl)-2-methyl-3-hydroxy-4-pyridone, and other moieties known in the art to chelate iron. Compounds which inhibit NO synthase activity are known in the art and include, *e.g.*, Nγ-substituted arginine analogs, especially of the L-configuration, including L-Nγ-nitro-arginine (a specific inhibitor of cerebral NO synthase), L-Nγ-amino-arginine, and L-Nγ-alkyl-arginines; or an ester thereof, preferably the methyl ester. Exemplary antioxidants include ascorbic acid, tocopherols including alpha-tocopherol, and the like.

Moreover, a linkage between, for example, a compound of the invention and a pharmacophore can be selected such that the two pharmacophores are cleaved from each other *in vivo* (*e.g.*, by the selection of a linkage which is labile *in vivo*). Examples of such biologically labile linkages are known in the art. *See, e.g.,* Silverman, cited above. Advantageously, such a "hybrid" drug can be designed to be transported within the body to reach a site or organ such as the brain, where one or more of the moieties exert a biological effect, at which site the hybrid drug can be cleaved to provide two active drug moieties.

In another embodiment, the invention provides a method for concurrently treating, preventing, or inhibiting epileptogenesis and ictogenesis, the method including the step of administering to a subject in need thereof a sufficient amount of a compound of the formula:



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

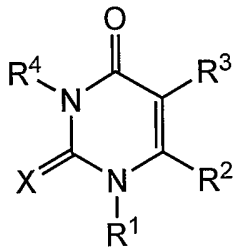
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -$CH_2CH(OH)$-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -$C{\equiv}CC_4H_9$, -$CH_2N(H)$-phenyl, -$CH_2N(H)CH_2$-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or pharmaceutically acceptable salts or esters thereof.

In another embodiment, the invention provides a method for treating, preventing, or inhibiting a disorder associated with NMDA receptor activity. The method includes the step of administering to a subject in need thereof a sufficient amount of a compound of the formula:



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or pharmaceutically acceptable salts or esters thereof.

Compounds which find use in the therapeutic methods of the invention can be determined through routine screening assays. For example, the animal model of Phase 1 epileptogenesis described in the Exemplification, *infra*, can be employed to determine whether a particular compound has anti-epileptogenic activity against Phase 1 epileptogenesis. Chronic epileptogenesis can be modeled in rats (and candidate compounds screened) with the kindling assay described by Silver *et al.* (*Ann. Neurol.* (1991) 29:356). Similarly, compounds useful as anticonvulsants can be screened in conventional animal models, such as the mouse model described in Horton, R.W. *et al.*, *Eur. J. Pharmacol.* (1979) 59:75-83. Compounds or pharmacophores useful for, *e.g.*, binding to or inhibition of receptors or enzymes can be screened according to

conventional methods known to the ordinarily skilled practitioner. For example, binding to the GABA uptake receptor can be quantified by the method of Ramsey *et al.* as modified by Schlewer (Schlewer, J., *et al.*, *J. Med. Chem.* (1991) 34:2547). Binding to the glycine site on an NMDA receptor can be quantified, *e.g.*, according to the method described in Kemp, A., *et al.*, *Proc. Natl. Acad. Sci. USA* (1988) 85:6547. Effect on the voltage-gated Na^+ channel can be evaluated *in vitro* by voltage clamp assay in rat hippocampal slices.

Assays suitable for screening candidate compounds for anticonvulsive and/or anti-epileptogenic activity in mice or rats are further described in the Exemplification, *infra*.

Another embodiment of the invention pertains to a method of treating, preventing, or inhibiting seizures in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of the present invention, such that seizures are treated, prevented, or inhibited. In a particular embodiment, the seizures are caused by epileptogenesis.

Another embodiment of the invention pertains to a method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated, prevented, or inhibited.

Another embodiment of the invention pertains to a method of treating, preventing, or inhibiting seizures in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of the present invention, such that seizures are treated, prevented, or inhibited, *e.g.*, wherein the seizures are caused by epileptogenesis.

Another embodiment of the invention pertains to a method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of the present invention, such that epileptogenesis is treated, prevented, or inhibited.

B. Characterization of Epilepsy

Neurons that are the most prone to epilepsy are primarily located in the cerebral cortex, which includes the frontal, parietal, occipital and temporal lobes, with the latter lobe appearing to be the most predisposed region of the brain to the process of epileptogenesis. A seizure focus may therefore be defined as that part of the brain from which seizures arise and eventually spread. The anatomical location of this focus inside the brain may determine the observed symptoms or the seizure type. Thus, for instance, when the focus is located in the motor cortex, the epileptic seizures may be primarily characterized by motor shaking of the body; while difficulties with speech are usually the first signs of seizures with seizure foci located in the temporal lobe region. Several examples of causes of epilepsy/seizures, without limitation, are described below.

Causes of epilepsy

Normal brain functions rely on electrical activities within the CNS that are maintained by a delicate balance between excitatory and inhibitory inputs to neurons. Many factors involving membrane receptors, ion channels, and carriers or transporters may affect this balance and cause abnormalities in neural electrical discharges, which could lead to CNS disorders such as epilepsy in case of neuronal hyperexcitability [5-7]. Undoubtedly, modern Neuroscience has made considerable progress in the understanding of this disorder, but still about 70% of epilepsy cases remain classified as idiopathic or, simply, not linked to a specific etiology [8].

Any infectious or physical trauma to the CNS is a potential cause for epilepsy. Prenatal trauma, congenital defects, birth trauma, *e.g.*, birth anoxia, cerebral palsy, and degenerative neurological diseases or postnatal insults, such as brain trauma, CNS infections, brain tumors, and cerebrovascular diseases greatly increase the incidence of epilepsy. In addition to trauma, metabolic or genetic abnormalities may also play a significant role in the predisposition to developing this disorder.

A deficiency in dihydropyrimidine dehydrogenase, a key enzyme in the metabolism of pyrimidine bases, is known to increase the level of uracil and thymine in body fluids and may have neurological implications in disorders such as epilepsy and mental retardation [10-12]. Recently, Woo et al. [13] showed that disruption of the

mouse gene (KCC2) encoding the neuronal-specific K-Cl cotransporter, which is thought to participate in the postnatal brain development of inhibitory mechanisms by driving intracellular chloride ions to low concentrations and shifting the reversal potential for chloride ion conductances, was associated with hyperexcitability and epilepsy. In fact, animals deficient in KCC2 exhibited frequent generalized seizures and died shortly after birth suggesting that these genes play a key role in controlling CNS excitability at birth as well as in adult life.

Classification of seizures

Epileptic seizures are very complex and their clinical manifestations may include an array of sensory, motor and autonomic phenomena. In an attempt to build a more comprehensive and systematic seizure classification, the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE) has developed a universally accepted seizure classification system based on a review of videotaped seizures at commission workshops and inputs from other working groups. This resulted in the 1964 ILAE seizure classification system [14], which was later revised in 1981 [15] to include electroencephalographic (EEG) data that are required to determine whether the initial neuronal activation is focal or generalized. The ILAE seizure classification system distinguishes three main categories of seizures, which are further divided in subcategories, and attempts to describe seizures with respect to six major aspects including, for example, clinical seizure type, electroencephalographic (EEG) seizure type, *i.e.* the EEG patterns during a clinical seizure, EEG interictal expression, *i.e.* EEG patterns between clinical seizures, anatomical substrate, etiology, and age.

Partial seizures

Partial seizures, also known as focal or local seizures, are the most common seizures occurring in about two-thirds of the epilepsy patient population. For these seizures, the first clinical and EEG changes indicate an initial activation of a system of neurons limited to one cerebral hemisphere. When consciousness (*i.e.* degree of awareness or responsiveness of the patient to externally applied stimuli) is impaired during the attack, the seizure is classified as a complex partial seizure; otherwise, it is a

simple partial seizure. However, simple partial seizures may evolve to complex partial seizures and/or generalized tonic-clonic convulsions, also called secondary generalized seizures.

Epilepsy patients suffering from complex partial seizures usually experience an impairment of consciousness and psychic symptoms that proceed from disturbance of higher cerebral functions. Simple partial seizures may involve motor signs of any portion of the body depending on the site of origin of the seizure attack in the motor strip in the cortex. These seizures may remain strictly focal or they may spread to contiguous cortical areas to produce a sequential involvement of body parts in an epileptic "march" leading to a seizure type known as the Jacksonian seizure. Simple partial seizures may also involve autonomic signs (vomiting, flushing, sweating, piloerection, pupil dilation) or somatosensory symptoms, which arise from the cortical areas subserving sensory functions. In these cases, patients may experience vertiginous symptoms (sensations of falling in space or floating); visual hallucinatory phenomena involving people or scenes; auditory sensations like music; olfactory sensations, usually in the form of unpleasant odors; gustatory sensations that may be odious or pleasant taste hallucinations varying in elaboration from crude (sweet, bitter, salty, or sour) to sophisticated.

Generalized seizures

Generalized seizures are primary generalized seizures that occur in about one-third of the epilepsy patient population [15]. They may be further divided into three major subcategories including, for example, absence seizures, myoclonic seizures and generalized tonic clonic seizures. The first clinical signs of generalized seizures indicate an involvement of both cerebral hemispheres. Their initial manifestations may include the impairment of consciousness, bilateral movements as well as bilateral ictal EEG patterns that reflect the extent of neuronal discharges in the two hemispheres.

Absence seizures

Abesence seizures, also known as "petit mal", are non-convulsive seizures that occur in childhood and usually tend to cease in late teenage years. The hallmark of their attack is a sudden onset, interruption of ongoing activities, a blank stare, possibly a brief upward rotation of eyes. The attack is usually brief, lasting about 30 seconds or less, and dissipating as quickly as it started. There are various forms of this seizure subcategory and a patient may experience one or a mixture of forms, including the three major subtypes of these seizures, *i.e.*, simple absence, Lennox-Gastaut Syndrome (LGS), and complex absence.

In circumstances wherein consciousness impairment is the only clinical sign during the seizure attack, it is a simple absence; otherwise, it may either be the Lennox-Gastaut Syndrome or the complex absence. The EEG of complex absences is characterized by three per second spikes and slow discharge during ictal periods. In LGS, the seizures are extremely brief and frequently incorporate many symptoms of simple and complex absences. The EEG for LGS is diffusely slow with typical spikes and wave discharges at frequencies other than 3 Hertz. Although rare, absence seizures may sometimes evolve into generalized tonic-clonic activity producing a seizure-type that is different from the complex partial seizures with secondary generalization.

Several examples of absence seizures include, for example, absence seizures with impairment of consciousness only (Simple absence), wherein no other activities, beside consciousness impairment, take place during the attack; absence seizures with mild clonic components, wherein the onset is indistinguishable from the above but clonic movements may occur in the eyelids, at the corner of the mouth or in other muscle groups with severity varying from almost imperceptible movements to generalized myoclonic jerks; absence seizures with atonic components, which is characterized by a diminution in tone of muscles sub-serving posture as well as limbs leading to drooping of the head, occasional slumping of the trunk, dropping of the arms, and relaxation of the grip (however, it is rare for the muscle tone to be so diminished that the patient falls down); absence seizures with tonic components, wherein tonic muscular contractions may occur during the seizure attack leading to increase in muscle tone that may affect the extensor or flexor muscles, and the head may tonically draw to one side or the other (if the patient is standing, the head can be drawn backward and the trunk may arch

leading to retropulsion); absence seizures with automatisms, wherein during the attack, purposeful or quasi-purposeful movements such as lip licking, chewing, swallowing, clothes fumbling, or aimless walking, may occur in the absence of awareness.

Myoclonic seizures

Myoclonic seizures are characterized by single or multiple quick involuntary jerks known as myoclonus or myoclonic jerks. These jerks are sudden, brief shock-like contractions, which may be generalized or confined to the face and trunk or to one or more extremities or even to individual muscles or groups of muscles. Myoclonic seizure attacks occur predominantly around the hours of going to sleep or awakening from sleep. However, it should be noted that in the absence of epilepsy, other health conditions including spinal cord disease, dyssynergia cerebellaris myoclonia, paramyoclonus multiplex, subcortical segmental myoclonus, and opsoclonus-myoclonus syndrome may present myoclonic jerks as symptoms.

Generalized tonic clonic seizures or "grand mal"

Generalized tonic clonic seizures or "grand mal" seizures occur in both childhood and adult life but they are not as frequent as absence seizures. Their frequency may vary from one a day, to one every three months, or even to one every few years. "Grand mal" is the most common type of generalized seizure. Although the majority of patients lose consciousness without any premonitory symptoms, some may experience a vague ill-described warning. During the attack, there is a sudden sharp tonic contraction of muscles and the patient falls to the ground in the tonic state, passing urine involuntarily and injuring his/her tongue, if it is caught between the teeth, as the jaw contracts. The tonic contraction inhibits respiration, possibly, leading to cyanosis (discoloration of the skin due to lack of oxygen in the blood). After this tonic phase, there next emerges a transition phase, which is marked by rapid vibratory tremors that progress to violent flexor contractions and relaxations. The clonic phase is characterized by convulsive movements and small gusts of grunting respiration within movement intervals. As the seizure approaches its end, the frequency of clonic contractions gradually decreases and saliva may froth from the mouth. Finally, deep respiration

occurs and all the muscles relax but the patient remains unconscious for several minutes, and then awakes feeling stiff and diffusely sore.

Unclassified epileptic seizures

This category includes all seizures that cannot be classified in either of the two previously described categories due to inadequate or incomplete data. It includes febrile seizures, status epilepticus, and neonatal seizures. Febrile seizures, usually, occur during the rising phase of a fever in children (age: six months to five years). Status epilepticus refers to repetitive seizures lasting more than 30 minutes without total recovery between attacks. This is most frequently observed in generalized seizure patients and constitutes a medical emergency that may result in brain damage. Many of the seizures occurring in infants and newborns including rhythmic eye movements, chewing, swimming movements, jittering and apnea are classified in this category.

III. Compounds for Use in the Methods of the Invention

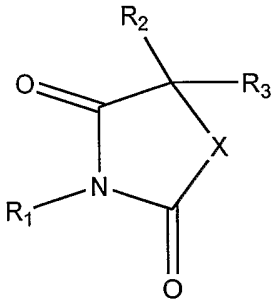
In one embodiment, the invention provides compounds useful for the treatment, prevention, or inhibition of seizure-associated states, including epileptogenesis and ictogenesis.

Molecules such as β-alanines that have neuroprotective properties may prevent primary and/or secondary epileptogenesis. For example, such molecules may prevent a focus from forming or prevent an existing focus from enlarging and worsening (*e.g.*, increasing in size or increasing in number) respectively. In this regard, they may be considered as not only anticonvulsants but also anti-epileptogenic drugs. Drugs developed for the treatment or management of epilepsy will be required to fulfill certain requirements in order to exert a therapeutic biological activity inside the brain. Due to the properties of the BBB, β-alanine and other charged molecules are difficult candidates for drug development in the therapy of neurological disorders. Fortunately, the prodrug approach may allow us to circumvent this problem using uracil analogs as they are metabolized *in vivo* to β-amino acids following the path illustrated below. However, in

one aspect, the invention is directed to prodrugs that are active to treat, prevent or inhibit seizure-associated states, *e.g.*, epileptogenesis or ictogenesis, prior to metabolization.

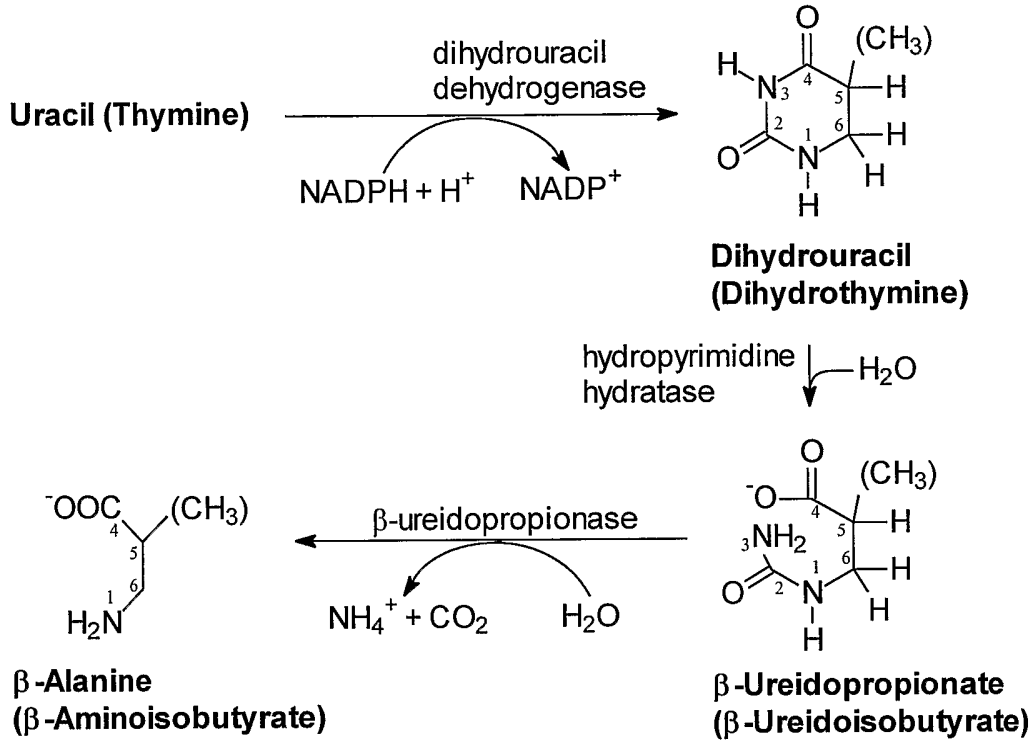
Examination of the uracil structure reveals a relation to a hydantoin moiety. Hydantoins are prototypic anticonvulsants, which bind Na+ channels. For convenience, the structure of the hydantoin moiety is depicted below

Structural formula of a hydantoin moiety.

	X
	NH

As the hydantoin pharmacophore is significantly related to the uracil compounds of the invention, using the "active" prodrug approach it is expected that the compounds of the present invention may also have anti-convulsant activity.

Accordingly, in one embodiment, uracil-based drugs may therefore be expected to exhibit dual biological activity; (1) as anticonvulsants mediating their biological effects *via* the voltage-gated sodium ion channels due to their similarity with hydantoins, and (2) as antiepileptogenic prodrugs that are anticipated to metabolize to β-alanine-based drugs. The enzymatic catabolism of uracils as illustrated below indicates that the C-4, C-5, C-6 and N-1 atoms of the uracil ring are preserved from the parent uracil to the corresponding β-amino acid.

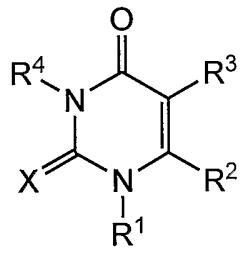


The use of substituted or unsubstituted uracils, and derivatives or analogs thereof, may be especially advantageous as certain uracil compounds have been found to have anti-ictogenic properties when tested in an anti-seizure model in rats. *See, e.g.,* *Medicinal Chemistry* Volume V; W. J. Close, L. Doub, M. A. Spielman; Editor W. H. Hartung; John Wiley and Sons (1961). Thus, the prodrug form of the compound, *i.e.*, a uracil, can have anti-seizure activity, while the metabolically-produced β-amino anionic compounds can have anti-epileptogenic and/or anti-ictogenic activity. These activities, individually and in combination, can provide effective therapy for seizure-associated states in mammals (including humans).

Additionally, it is significant to note that pyrimidine compounds, such as 5-fluorouracil (5FU), have previously been used as anti-neoplastic agents. The anti-cancer activity of 5FU and similar compounds is believed to be due to a "suicide substrate" mechanism where the 5FU inhibits thymidylate synthase, an enzyme important in DNA synthesis. Accordingly, in particular embodiments, pyrimidine compounds administered according to the invention for the treatment, prevention, or inhibition of seizure-associated states (*e.g.*, inhibition of epileptogenesis) do not significantly inhibit thymidylate synthase. Without wishing to be bound by theory, it is believed that

inhibition of thymidylate synthase by pyrimidine compounds is increased by the presence of electronegative groups at the 5-position of the pyrimidine ring, and can therefore be decreased by providing such compounds with non-electronegative groups at the 5-position of the pyrimidine ring. It is further believed that by providing substituents with sufficient steric bulk to decrease the ability of the pyrimidine compound to bind to thymidylate synthase, inhibition of thymidylate synthase can be decreased.

In one embodiment, the compounds of the present invention can be represented by the formula



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

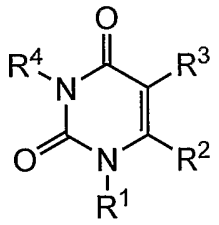
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments of the invention, at least one of R^1, R^2, R^3, or R^4 is not hydrogen, *e.g.*, at least two of R^1, R^2, R^3, or R^4 are not hydrogen, *e.g.*, at least three of R^1, R^2, R^3, or R^4 are not hydrogen, *e.g.*, wherein R^1, R^2, R^3, and R^4 are not hydrogen. In certain embodiments, R^4 is not hydrogen.

In a particular embodiment, the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

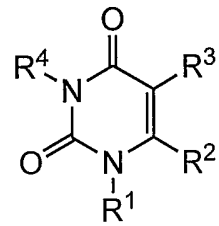
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments, R^1, R^2, and R^4 are not H, while R^3 is H, Br, I, -C≡CC_4H_9, or CH_3. In certain embodiments, R^1 and R^4 are not H, while R^2 is CH_3, and R^3 is H or Br. In certain embodiments, R^1 and R^4 are not H, while R^2 and R^3 are CH_3. In certain embodiments, R^2 and R^4 are not H, while R^3 is Br, and R^1 is benzyl or isopropyl. In certain embodiments, R^3 and R^4 are not H, while R^2 is H or CH_3, and R^1 is benzyl. In certain embodiments, R^2 is not H, while R^1 is benzyl, R^3 is -C≡CC_4H_9, and R^4 is H or benzyl. In certain embodiments, R^2 is not H, while R^1 and R^4 is p-NO_2-benzyl, and R^3 is Br. In certain embodiments, R^2 is not CH_3, while R^1 and R^4 is p-NO_2-benzyl, and R^3 is H.

In another particular embodiment, the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl;

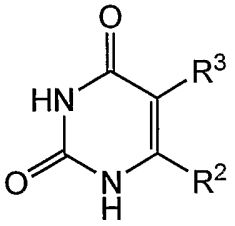
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, and n-butyl;

R^3 is selected from the group consisting of H, Br, I, and -C≡CC_4H_9; and

R^4 is selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments, R^1, R^2, and R^4 are not H, while R^3 is H, Br, I, or -C≡CC_4H_9. In certain embodiments, R^1 and R^4 are not H, while R^2 is CH_3, and R^3 is H or Br. In certain embodiments, R^2 and R^4 are not H, while R^3 is Br, and R^1 is benzyl. In certain embodiments, R^3 and R^4 are not H, while R^2 is H or CH_3, and R^1 is benzyl. In certain embodiments, R^2 is not H, while R^1 is benzyl, R^3 is -C≡CC_4H_9, and R^4 is H or benzyl. In certain embodiments, R^2 is not H, while R^1 and R^4 is p-NO_2-benzyl, and R^3 is Br. In certain embodiments, R^2 is not CH_3, while R^1 and R^4 is p-NO_2-benzyl, and R^3 is H.

Another particular embodiment of the compound is



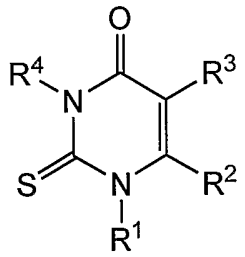
wherein

R^2 is selected from the group consisting of H, phenyl, benzyl, and -CH_2CH(OH)-phenyl; and

R^3 is selected from the group consisting of H, Br, -CH_2N(H)-phenyl, and -CH_2-O-benzyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments, R^2 is not H, while R^3 is H or Br. In certain embodiments, R^2 is not H, while R^3 is Br.

In yet another particular embodiment, the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

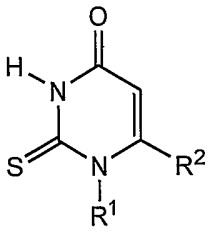
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3,-C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof.

In yet another particular embodiment, the compound is



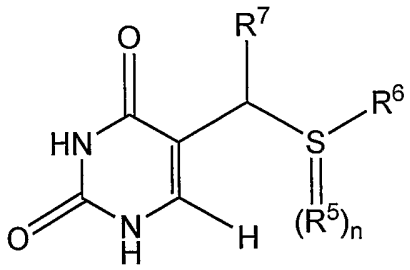
wherein

R^1 is selected from the group consisting of H and -CH_2SCH_3; and

R^2 is selected from the group consisting of H, n-butyl, ethyl, n-propyl, phenyl, and benzyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof.

In yet another particular embodiment, the compound is



wherein

n is 0 or 1;

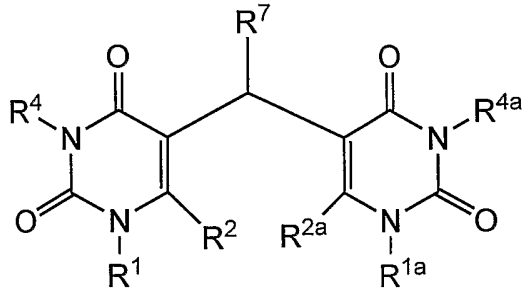
R^5 is O or absent;

R^6 is selected from the group consisting of CH_3 and benzyl; and

R^7 is selected from the group consisting of H and phenyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments, R^7 is not H, while R^6 is CH_3 and R^5 is absent.

In yet another particular embodiment, the compound is



wherein

R^1 and R^{1a} are independently selected from the group consisting of H, benzyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

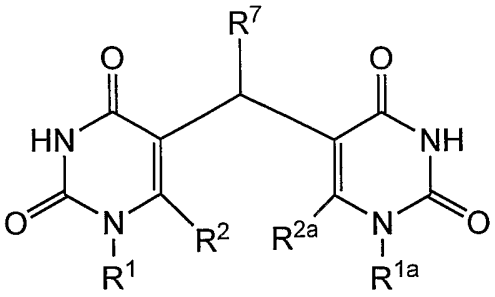
R^2 and R^{2a} are independently selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^4 and R^{4a} are independently selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl; and

R^7 is selected from the group consisting of H and phenyl;

or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof. In certain embodiments, R^1, R^{1a}, R^4 and R^{4a} are not all benzyl.

In yet another particular embodiment, the compound is



wherein

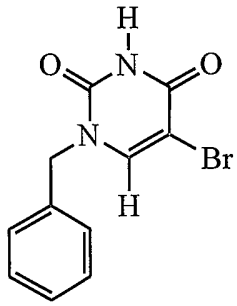
R^1 and R^{1a} are independently selected from the group consisting of H and benzyl;

R^2 and R^{2a} are independently selected from the group consisting of H, CH_3, and benzyl; and

R^7 is selected from the group consisting of H and phenyl;

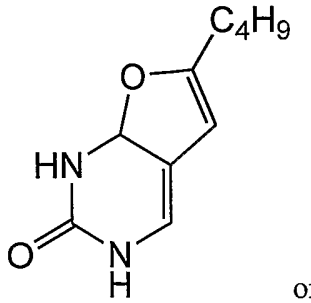
or acceptable salts, *e.g.*, pharmaceutically acceptable salts, or esters thereof.

Several specific compounds of the invention are listed below as compounds 1-80 and are intended to be considered independently. For example, in one specific embodiment, the compound is



or an acceptable salt, *e.g.*, pharmaceutically acceptable salt, thereof.

In another specific embodiment, the compound is



or an acceptable salt, *e.g.*, pharmaceutically acceptable salt, thereof.

In certain embodiments, the compound of the invention is not a compound of published U.S. Utility Application, publication number 20030153584-A1, the contents of which are hereby expressly incorporated herein by reference in its entirety. In particular embodiments, the compound of the invention is not compound 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 32, 38, 47, 61, 71, 74, or 76 as depicted in the Exemplification section (*i.e.*, wherein the exclusion of each compound listed is intended to be considered as a separate embodiment).

In an additional embodiment, the compounds of the invention are selected based on at least one of the biological assays described herein. In yet another embodiment, the compounds of the invention are selected based on at least two of the biological assays described herein. In yet another embodiment, the compounds of the invention are selected based on at least three of the biological assays described herein. In yet another embodiment, the compounds of the invention are selected based on at least four of the biological assays described herein. It should be understood that, based on the distinctions among the biological assays described herein, a compound of the present invention is useful in the methods of the present invention if the compound produces a positive result within at least one of these biological assays.

Moreover, such analysis may include a ratios, *e.g.*, therapeutic ratios, of data obtained within a single assay, *e.g.*, % protective effect (*e.g.*, greater than a 50% protective effect, wherein protective effect may be defined in one embodiment as seizure onset delay multiplied by 100% and divided by 180), or between the data obtained from two or more different assays. In particular embodiments, the selection may be based on % protective effect, and the results of the PTZ induced seizure model, MES-seizure model, and the neurotoxicity screen. Additionally, in certain embodiments, the compound of the invention is not neurotoxic

Accordingly, in one embodiment the invention is directed to a method of treating, preventing, or inhibiting a seizure-associated state, *e.g.*, epileptogenesis or ictogenesis, in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of the present invention, wherein the compound is not neurotoxic, such that the seizure-associated state is treated, prevented, or inhibited.

The present invention is further directed, in particular embodiment to the use of prodrugs which are converted *in vivo* to the therapeutic compounds of the invention. Such prodrugs can be used to alter the biodistribution (*e.g.*, to allow compounds which would not typically cross the blood-brain barrier to cross the blood-brain barrier) or the pharmacokinetics of the therapeutic compound. For example, an anionic group, *e.g.*, a carboxylate or sulfonate, can be esterified, *e.g.*, with a methyl group or a phenyl group, to yield a carboxylate or sulfonate ester. When the carboxylate or sulfonate ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, to reveal the anionic group. Such an ester can be cyclic, *e.g.*, a lactone or sultone, or two or more anionic moieties may be esterified through a linking group. An anionic group can be esterified with moieties (*e.g.*, acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound. Alternatively, an anionic moiety can be esterified to a group which is actively transported *in vivo*, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the therapeutic moieties to particular organs. In another embodiment, the prodrug is a reduced form of an anionic group, *e.g.*, a carboxylate or sulfonate, *e.g.*, an alcohol or thiol, which is oxidized *in vivo* to the therapeutic compound.

Furthermore, compounds of the present invention can be prepared according to a variety of synthetic procedures, some of which are known in the art. However, all novel syntheses, novel compounds, or novel pharmaceutical compositions described herein are intended to be included within the scope of the present invention. Moreover, exemplary syntheses are shown in the Exemplification section.

In particular, an additional embodiment of the invention is directed to the methods of preparation of compounds of the invention in accordance with the synthetic preparations described herein, *e.g.*, in the Exemplification section. For example, the scope of the invention includes novel reactions described herein, which may include, without intending to be limiting, reactions of uracil and benzyl bromide, synthesis of 6-substituted uracils or thiouracils *via* cyclization, reaction of 6-methyluracil and p-nitrobenzyl bromide, coupling reactions of N-mono- and N-di-benzyl-5-iodouracil and hexyne, bromination of 6-methyl-3-para-nitrobenzyluracil, iodination of 6-methyl-1, 3-bis-para-nitrobenzyluracil, bromination of 6-methyluracil, benzoylation reactions,

alkylation of benzoyl-protected uracil analogs, or synthesis of biuracil analogs *via* addition of sulfides and elimination of mercaptans. Furthermore, the scope of the present invention also includes the extension of the reactions described herein to other systems, *e.g.*, indoles and pyrroles, which contain the enamine moiety.

Compounds useful in the present invention may also include carrier or targeting moieties which allow the therapeutic compound to be selectively delivered to a target organ or organs. For example, if delivery of a therapeutic compound to the brain is desired, the compound may include a moiety capable of targeting the compound to the brain, by either active or passive transport (a "targeting moiety"). Illustratively, the carrier molecule may include a redox moiety, as described in, for example, U.S. Patent Nos. 4,540,564 and 5,389,623. These patents disclose drugs linked to dihydropyridine moieties which can enter the brain, where they are oxidized to a charged pyridinium species which is trapped in the brain. Thus, drug accumulates in the brain. Other carrier moieties include compounds, such as amino acids or thyroxine, which can be passively or actively transported *in vivo*. Such a carrier moiety can be metabolically removed *in vivo*, or can remain intact as part of an active compound. Many targeting moieties are known, and include, for example, asialoglycoproteins (*see, e.g.*, U.S. Patent No. 5,166,320) and other ligands which are transported into cells *via* receptor-mediated endocytosis.

The targeting and prodrug strategies described above can be combined to produce a compound that can be transported as a prodrug to a desired site of action and then unmasked to reveal an active compound.

IV. Pharmaceutical Compositions

Any of the compounds described herein may be formulated in combination with a pharmaceutically acceptable carrier. For example, an anti-ictogenic and/or anti-epileptogenic pharmaceutical composition of the present invention can include a sufficient amount of one or more of the compounds described herein effective to inhibit a seizure-associated state, such as epilepsy in a subject in need thereof, and a pharmaceutically acceptable carrier. Ictogenesis may be treated, prevented, or inhibited

in a subject in need thereof by administering to the subject a sufficient amount of one or more of the compounds described herein, such that ictogenesis is treated, prevented, or inhibited in the subject. In another aspect, epileptogenesis may be treated, prevented, or inhibited in a subject in need thereof by administering to the subject a sufficient amount of one or more of the compounds described herein, such that epileptogenesis is treated, prevented, or inhibited in the subject.

In one embodiment, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compounds described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. The pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) transdermal application, for example, *via* a "patch," for example, using a lotion, cream, solution, gel or solid ; or (5) intravaginally or intrarectally, for example, as a pessary, cream or foam. In a particular embodiment, the therapeutic compound is administered orally. The compounds of the invention can be formulated as pharmaceutical compositions for administration to a subject, *e.g.*, a mammal, including a human.

In another embodiment, the invention pertains to a pharmaceutical composition comprising a sufficient amount of a compound of the present invention to treat, prevent, or inhibit a seizure-associated state, *e.g.*, epilepsy, in a subject in need thereof, and a pharmaceutically acceptable carrier. In a particular embodiment, the pharmaceutical composition comprises a compound that is anti-ictogenic and/or anti-epileptogenic.

In a further embodiment, the invention further provides a kit which includes a container of a compound of the invention and instructions for administering a sufficient amount of the compound to a subject in need thereof such that a seizure-associated state (*e.g.*, epileptogenesis) is treated, prevented, or inhibited in the subject. Moreover, the kits of the invention provide convenient means for administering the compounds of the

invention. In a particular embodiment, the kit includes a sufficient amount of the compound, *e.g.*, in unit dosage form.

In another embodiment, the invention is directed to a kit for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that the seizure-associated state is treated, prevented, or inhibited.

In another embodiment, the invention is directed to a packaged pharmaceutical composition for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting the seizure-associated state in a subject.

In yet another embodiment, the invention is directed to a kit for treating, preventing, or inhibiting ictogenesis in a subject comprising a compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that ictogenesis is treated, prevented, or inhibited.

In another embodiment, the invention pertains to a kit for treating, preventing, or inhibiting epileptogenesis in a subject comprising said compound of the present invention, and instructions for administering a sufficient amount of the compound to a subject, such that epileptogenesis is treated, prevented, or inhibited.

Another embodiment of the invention is a packaged pharmaceutical composition for treating, preventing, or inhibiting ictogenesis in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting ictogenesis in a subject.

In an addition embodiment, the invention is directed to a packaged pharmaceutical composition for treating, preventing, or inhibiting epileptogenesis in a subject comprising a container holding a compound of the present invention; and instructions for using the compound for treating, preventing, or inhibiting epileptogenesis in a subject.

Furthermore, the compounds of the invention may be administered to subjects in a biologically compatible form, suitable for pharmaceutical administration *in vivo*. The language "biologically compatible form, suitable for administration *in vivo*" describes a

compound to be administered where any toxic effects are outweighed by the therapeutic effects of the compound. Administration of a therapeutically active amount of the therapeutic compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a compound of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of compound to elicit a desired response in the individual. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active compound may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

A compound of the invention can be administered to a subject in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with a material to prevent its inactivation. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Strejan *et al.*, (1984) *J. Neuroimmunol* 7:27). The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved

against the contaminating action of microorganisms such as bacteria and fungi. The pharmaceutically acceptable carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When the active compound is suitably protected, as described above, the composition may be orally administered, for example, with an inert diluent or an assimilable edible carrier. As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is within the scope of the present invention. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the therapeutic treatment of individuals.

V. Libraries

In another aspect, the invention provides libraries of compounds of the invention, and methods of preparing such libraries.

The synthesis of combinatorial libraries is well known in the art and has been reviewed (*see, e.g.*, Gordon *et al.*, *J. Med. Chem.* 37:1385-1401 (1994)). Thus, the invention includes methods for synthesis of combinatorial libraries of compounds of the invention. Such libraries can be synthesized according to a variety of methods. For example, a "split-pool" strategy can be implemented to produce a library of compounds. The library of immobilized compounds can then be washed to remove impurities. In certain embodiments, the immobilized compounds can be cleaved from the solid support to yield a compound of the present invention.

In another illustrative method of combinatorial synthesis, a "diversomer library" is created by the method of Hobbs, DeWitt *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 90:6909 (1993)). After creation of the library of compounds, purification and workup yields a soluble library of substituted compounds of the present invention.

Other synthesis methods, including the "tea-bag" technique of Houghten et al., *Nature* 354:84-86 (1991), can also be used to synthesize libraries of compounds according to the subject invention.

Combinatorial libraries can be screened to determine whether any members of the library have a desired activity, and, if so, to identify the active species. Methods of screening combinatorial libraries have been described (*see, e.g.*, Gordon *et al.*, *J Med. Chem.*, *op. cit.*). Soluble compound libraries can be screened by affinity chromatography with an appropriate receptor to isolate ligands for the receptor, followed by identification of the isolated ligands by conventional techniques (*e.g.*, mass spectrometry, NMR, and the like). Immobilized compounds can be screened by contacting the compounds with a soluble receptor; preferably, the soluble receptor is conjugated to a label (*e.g.*, fluorophores, colorimetric enzymes, radioisotopes, luminescent compounds, and the like) that can be detected to indicate ligand binding. Alternatively, immobilized compounds can be selectively released and allowed to diffuse through a membrane to interact with a receptor. Exemplary assays useful for screening the libraries of the invention are known in the art (*see, e.g.*, Gordon *et al.*, *J. Med. Chem.* 37:1385-1401 (1994)).

Combinatorial libraries of compounds can also be synthesized with "tags" to encode the identity of each member of the library. *See, e.g.,* U.S. Patent No. 5,565,324 and PCT Publication No. WO 94/08051). In general, this method features the use of inert, but readily detectable, tags, that are attached to the solid support or to the compounds. When an active compound is detected such as by one of the techniques described above, the identity of the compound is determined by identification of the unique accompanying tag. This tagging method permits the synthesis of large libraries of compounds which can be identified at very low levels.

In particular embodiments, the libraries of compounds of the invention contain at least 30 compounds, more preferably at least 100 compounds, and still more preferably at least 500 compounds. In particular embodiments, the libraries of compounds of the invention contain fewer than 10^9 compounds, more preferably fewer than 10^8 compounds, and still more preferably fewer than 10^7 compounds.

A library of compounds is preferably substantially pure, *i.e.*, substantially free of compounds other than the intended products, *e.g.*, members of the library. In particular embodiments, the purity of a library produced according to the methods of the invention is at least about 50%, more preferably at least about 70%, still more preferably at least about 90%, and most preferably at least about 95%.

The libraries of the invention can be prepared as described herein. In general, at least one starting material used for synthesis of the libraries of the invention is provided as a variegated population. The term "variegated population", as used herein, refers to a population including at least two different chemical entities, *e.g.*, of different chemical structure. For example, a "variegated population" of compounds of the present invention would comprise at least two different compounds of the present invention. Use of a variegated population of linkers to immobilize compounds to the solid support can produce a variety of compounds upon cleavage of the linkers.

Libraries of the invention are useful for, *inter alia,* drug discovery. For example, a library of the invention can be screened to determine whether the library includes compounds having a pre-selected activity, *e.g.*, anti-epileptogenic or anticonvulsant activity.

EXEMPLIFICATION

General Methods

Chemicals used as starting reagents were purchased from Aldrich Chemical Company and used with no further purification unless otherwise stated in the experimental section. All reactions were executed under a nitrogen or argon atmosphere unless otherwise specified in the synthetic procedures. All the compound structures and purity were confirmed by thin layer chromatography, high resolution mass spectrometry, and nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C). The reported yields are those of pure isolated products.

Thin layer chromatography (TLC) was performed on pre-coated Brinkmann silica gel 60 F254 plates with aluminum backing. Resolved TLCs were visualized with iodine vapor and UV light. Melting points (MP) were obtained using a MelTemp II capillary apparatus, and the values are uncorrected. The proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at various frequencies with Bruker Avance AC-250, AC-300 or AC-400 spectrometers. ^{1}H and ^{13}C chemical shifts are reported as δ values in parts per millions (ppm) relative to tetramethylsilane (TMS). Signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). ^{1}H NMR coupling constants are reported in Hertz (Hz) and refer to the apparent multiplicities as given on spectra. Molecular masses were obtained from spectra recorded on a VG Quattro Mass spectrometer with either electron impact (EI) or chemical ionization (CI). Reported yields are those of isolated pure products.

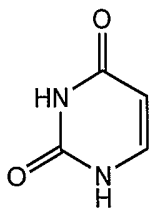
Experiments were performed using measurements, *e.g.*, weights or volumes, which were measured within experimental error. As such, measurements listed in the Exemplification are within the experimental uncertainty associated with the device used for the measurement, determination of which is art-recognized as being within the scope of the ordinarily skilled artisan.

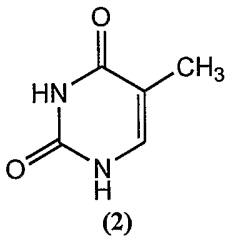
The following particular compounds of the invention were synthesized according to the methods described herein: 6-substituted uracils or thiouracils, 5-bromo-1, 3-dibenzyluracil, 6-methyl-1, 3-bis-para-nitrobenzyluracil, 5-bromo-1-para-nitrobenzyluracil, 5-bromo-6-(2-hydroxy-2-phenylethyl)uracil, 5-(hydroxymethyl)uracil and 5-(bromomethyl)uracil, 5-(benzyloxymethyl)uracil, 5-(benzylthiomethyl)uracil, 5-

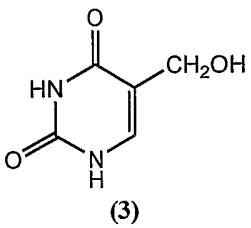
(phenylaminomethyl)uracil, 3-benzoyl-5-bromo-1-para-nitrobenzyluracil, 1,3-dibenzoyl-5-bromo-6-methyluracil, 3-benzoyluracil, 5-iodo-6-methyl-1,3-bis-para-nitrobenzyluracil, 1,3-dibenzyl-5-(hex-1-ynyl)pyrimidine-2,4(1H,3H)-dione, 6-methyl-3-para-nitrobenzyluracil,

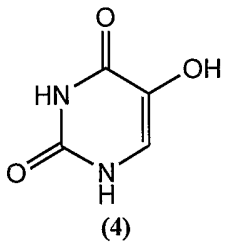
6-methyl-1,3-bis-para-nitrobenzyluracil, 5-bromo-6-methyl-3-para-nitrobenzyluracil, 3-benzoyl-5-bromo-6-methyluracil, 3-benzoyl-1-para-nitrobenzyluracil, 3-benzoyl-1-benzyl-5-iodouracil, 3-benzoyl-1-benzyluracil, 5-bromo-6-(2-hydroxy-2-phenylethyl)uracil, 5-(phenylaminomethyl)uracil, and as further described in the Exemplification section.

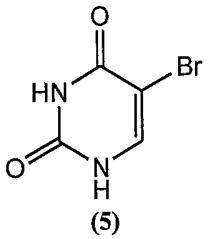
Particular compounds of the invention also include the following compounds, numbered 1 -80, each of which is considered a separate embodiment of the invention. Moreover, the listing of the compounds below is intended merely as a convenience, and not necessarily as imparting the characteristic of a group. Analogues of the following compounds are also within the scope of the present invention, including acceptable salts, *e.g.*, pharmaceutically acceptable salts, and esters thereof, *i.e.*, wherein the ester group may be added as a substituent to any position available for substitution and that would not affect the ability of the compound to perform its intended function.

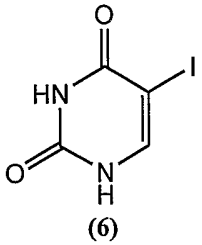

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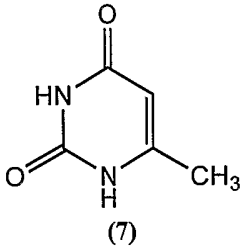

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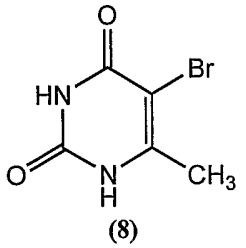

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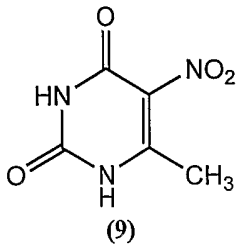

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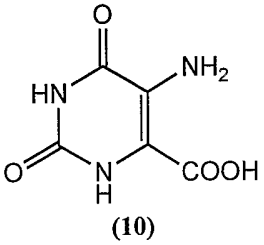

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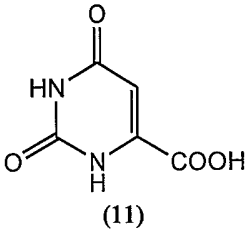

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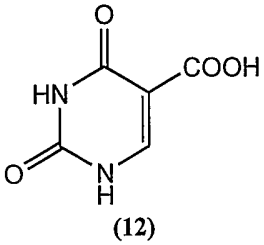

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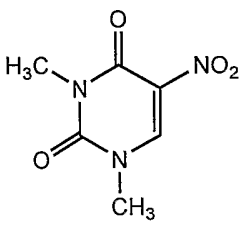

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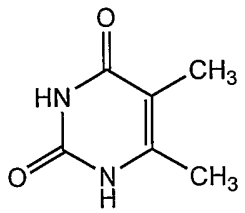

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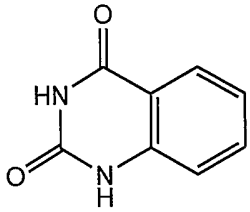

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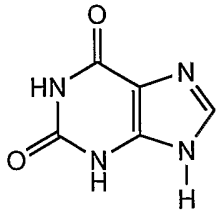
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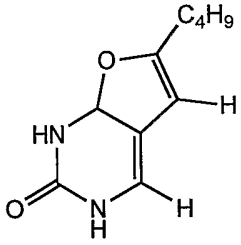
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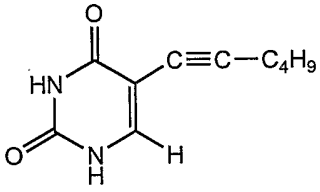
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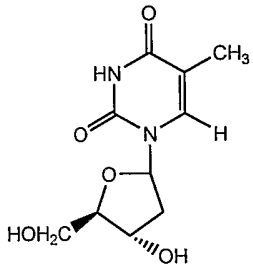
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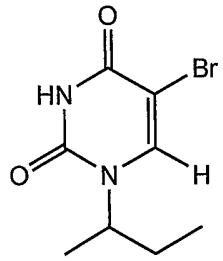
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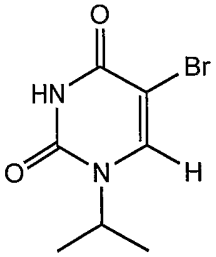
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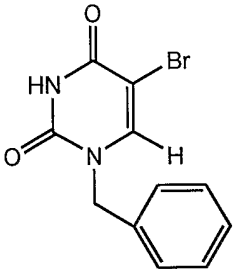
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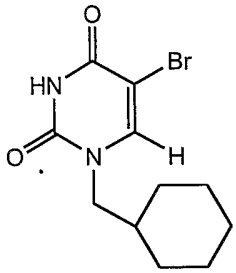
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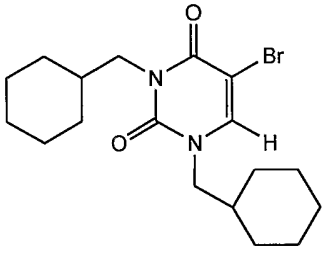
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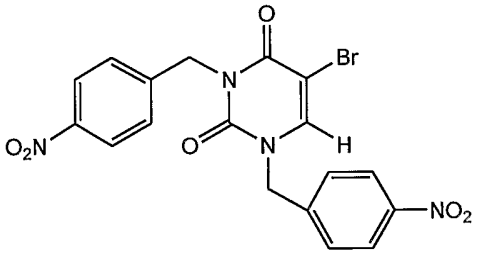
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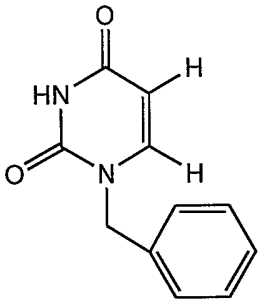
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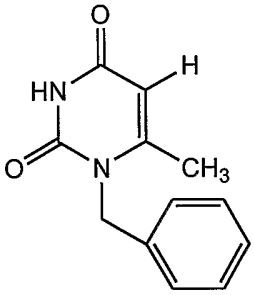
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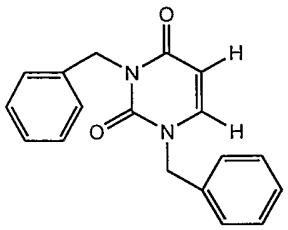
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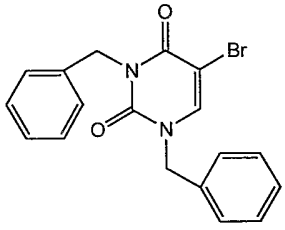
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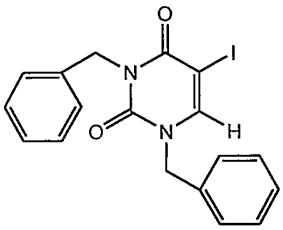
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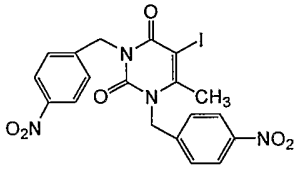
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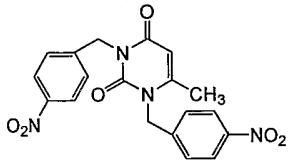
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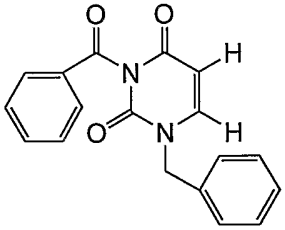
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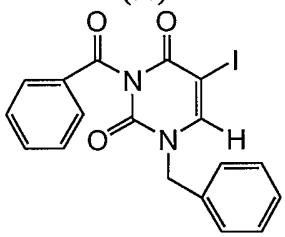
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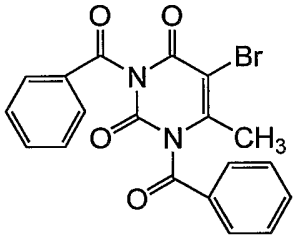
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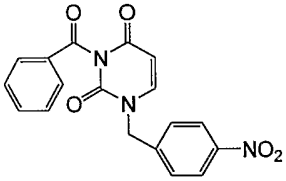
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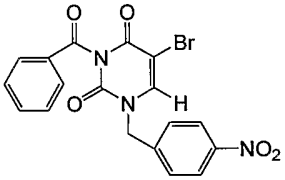
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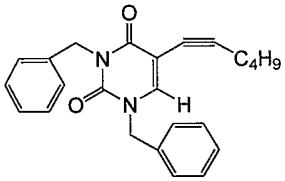
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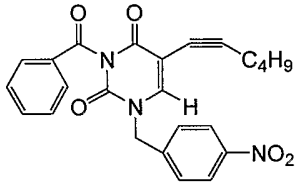
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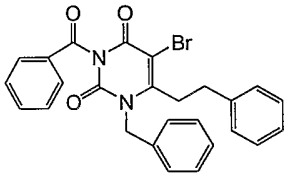
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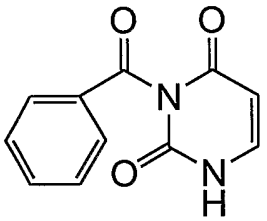
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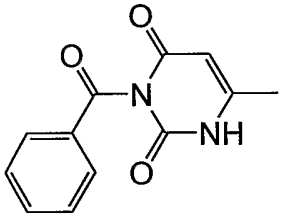
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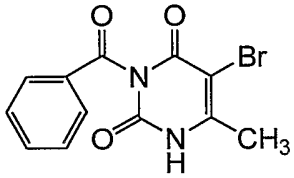
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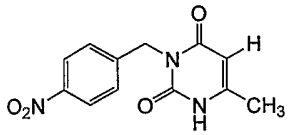
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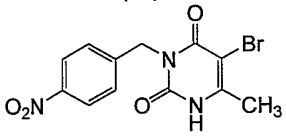
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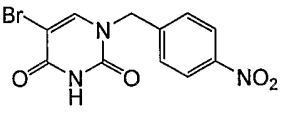
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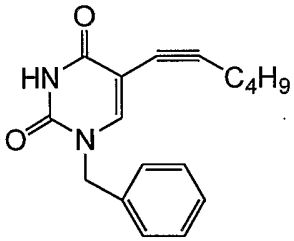
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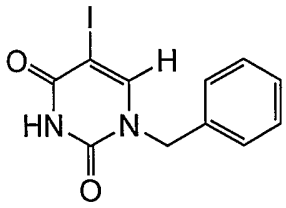
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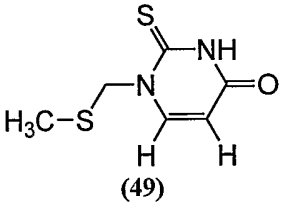
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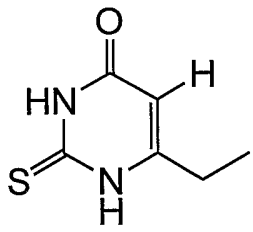
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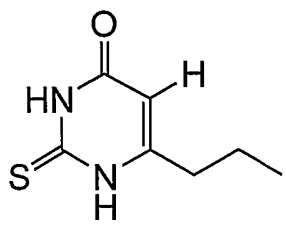
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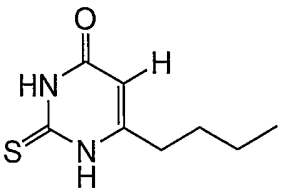
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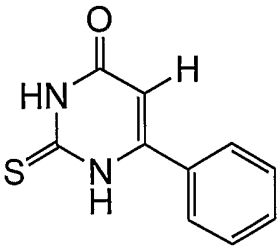
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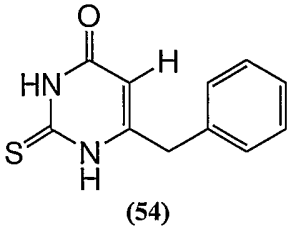
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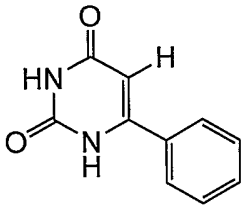
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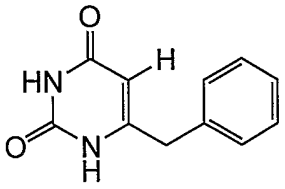
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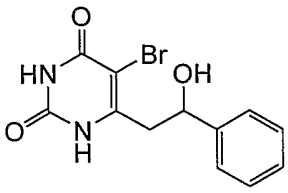
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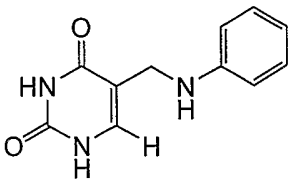
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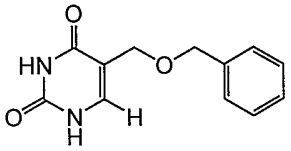
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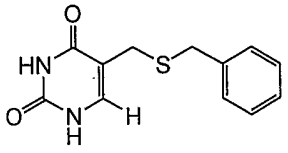
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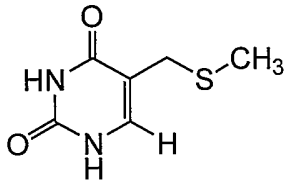
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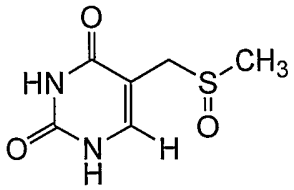
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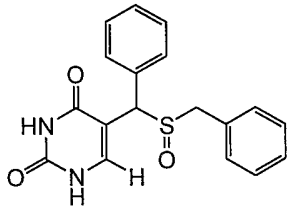
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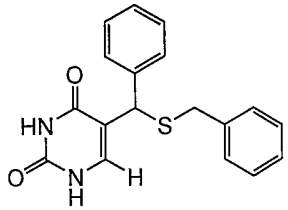
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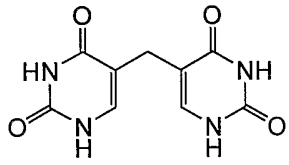
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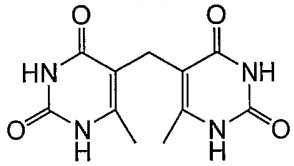
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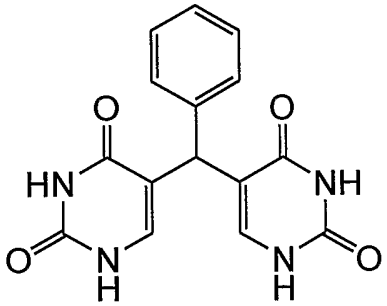
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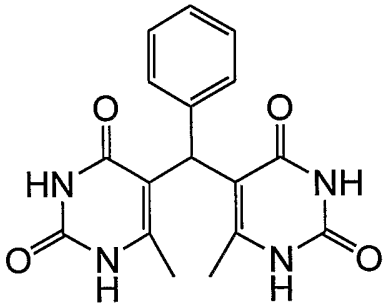
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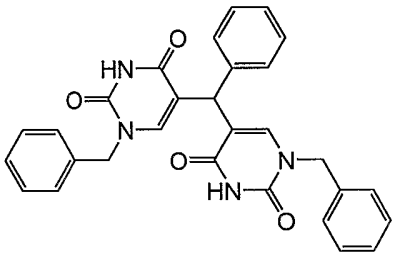
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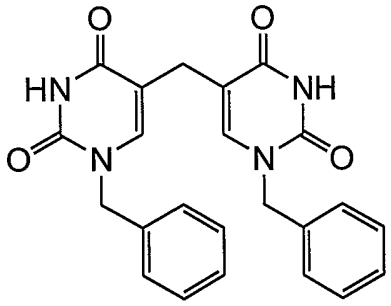
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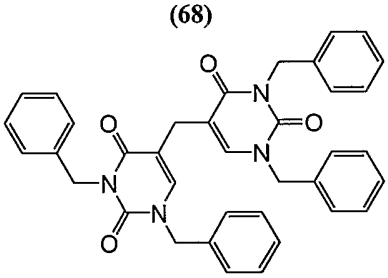
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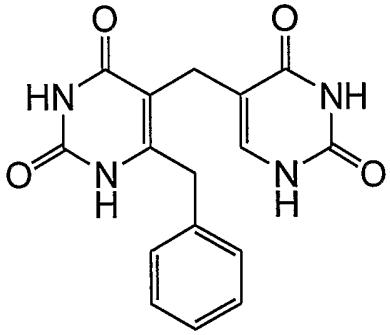
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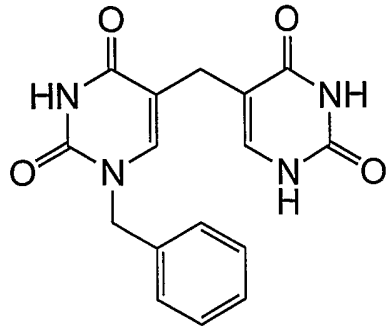
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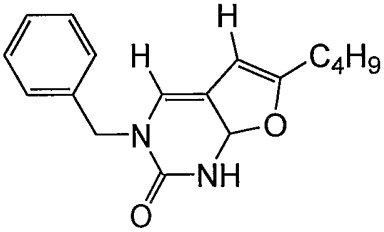
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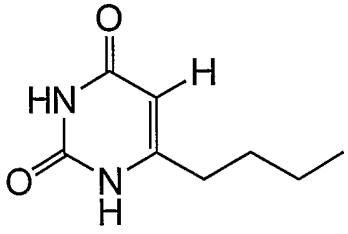
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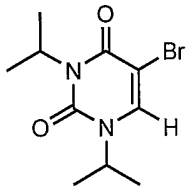
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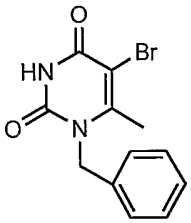
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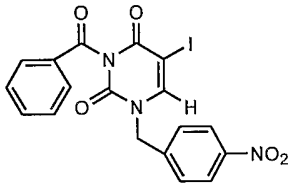
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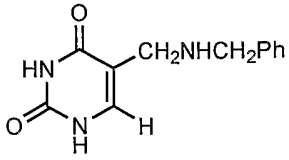
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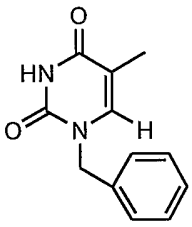
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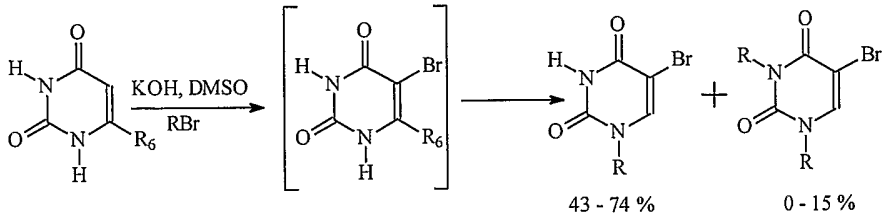
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Example 1

Reactions of uracil and benzyl bromide

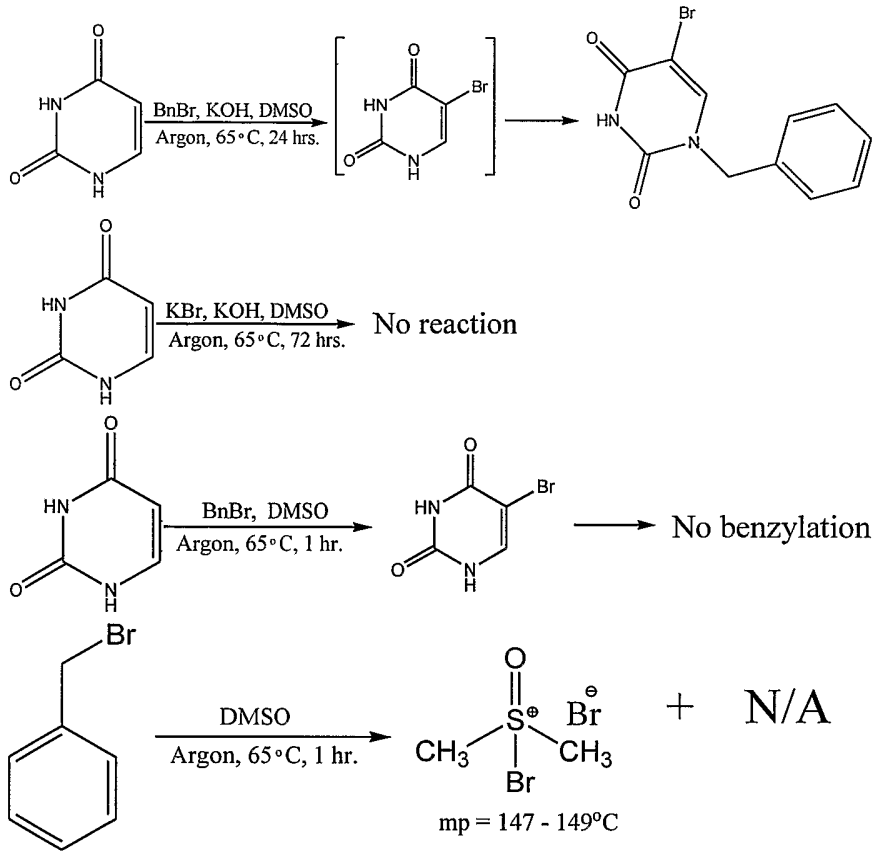
A series of 1-alkyl-5-bromouracils were prepared in a one pot-synthesis by direct reaction between alkyl bromides and uracil under basic conditions in DMSO
10 solvent only at 85°C. Under the same reaction conditions, when other solvents such as ethanol, acetonitrile, THF or DMF are used, the regioselectivity is lost, and no bromination is observed. Moreover, in attempt to understand the role of DMSO and the mechanism that lead to such bromination and regioselectivity the following reactions (equations 2 - 5) were performed and analyzed.



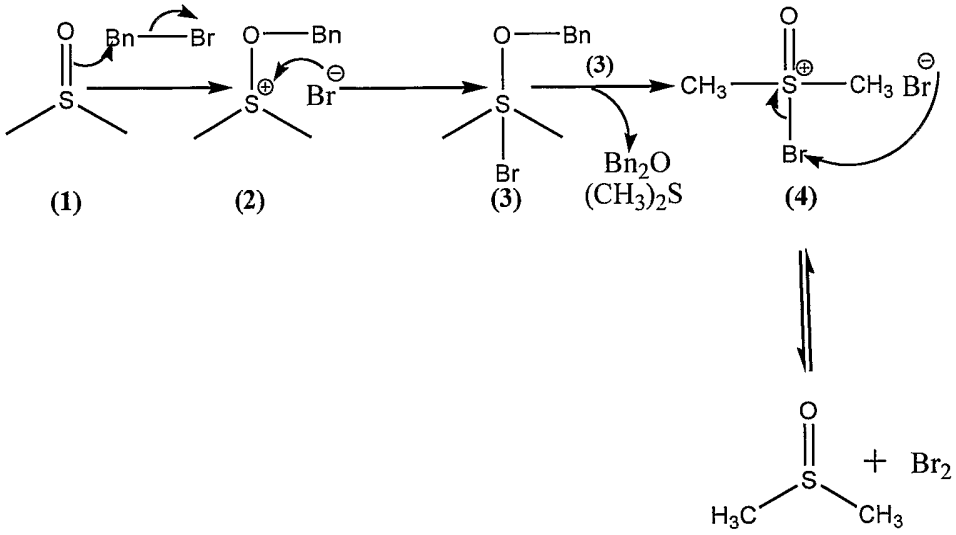
(1)

R = Benzyl, Isopropyl, Isobutyl, cyclohexylmethyl, p-nitrobenzyl
R_6 = H, CH_3

Note: Alkyl iodides lead to uncontrollable production of iodine; no alkylated product was isolated
Alkyl chlorides yield only alkylated products without halogenation



From these observations, and without wishing to be bound by theory, in one embodiment, the mechanism in the scheme shown below suggests that the bromodimethylsulfoxonium salt in equation **(5)** is the predominant brominating agent while the benzylating agent may primarily be the intermediate **(3)** in order to account for N-1 alkylation preferences for C-6 unsubstituted uracils. In this regard, it should be noted that 6-methyluracil, under these reaction conditions, undergoes bromination but no alkylated product is formed. However, the alkyl halide or the molecular bromine cannot be completely excluded as possibilities in the alkylation or bromination reactions since, in some instances, N-disubstituted uracils are obtained as minor products.

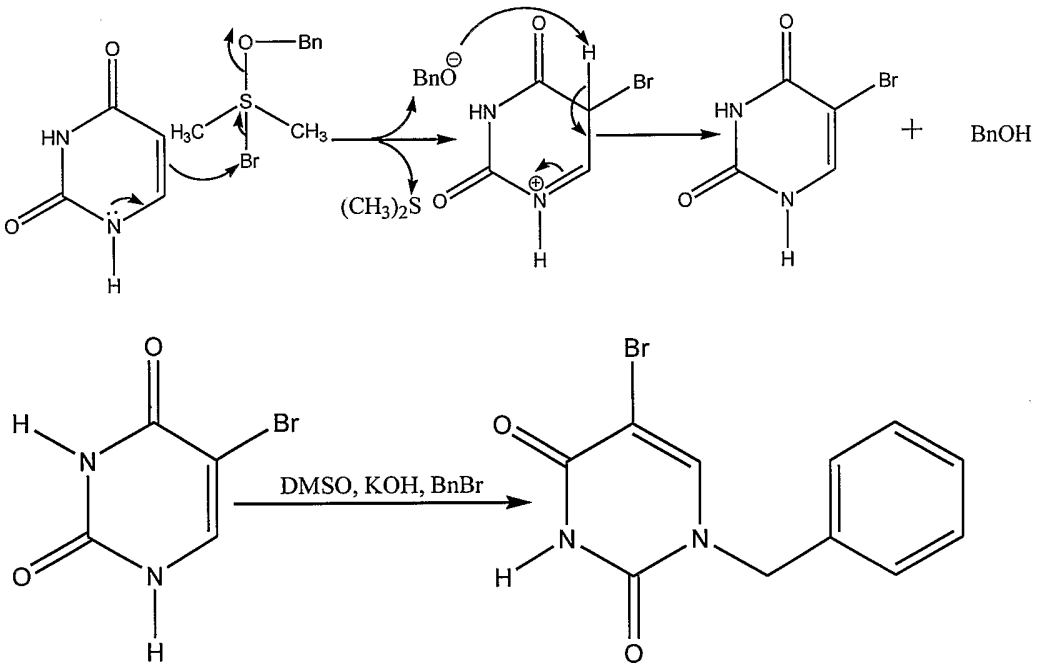


Example 2

Synthesis of bromodimethylsulfoxonium bromide

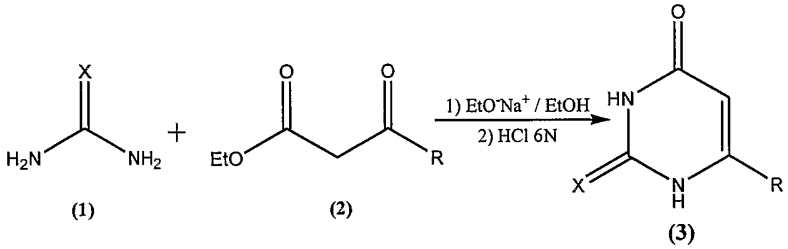
A clear solution of benzyl bromide (3.0 mL or 25.2 mmol) in dry DMSO (5.0 mL) was heated under an argon atmosphere in an oil bath maintained at 85°C. After 8 hours of vigorous stirring, the reaction mixture was cooled down to room temperature, and diluted with 20.0 mL of acetone. The mixture was stirred for additional 15 minutes and allowed to stand for crystallization. After filtration, the product was washed with acetone, then ether and dried overnight *in vacuo* to yield the pure product as white needle-like fine crystals (2.65g, 88.3%). Mp 147 – 149°C; proton NMR (250 MHz, DMSO d_6): δ 2.91 ppm (s, CH_3) and ^{13}C NMR (Jmod): δ 26.1 ppm. Megyeri and Keve claim to have synthesized this salt from dimethyl sulfoxide and bromotrimethyl-silane in hexane at room temperature but their product was an oil ^1H NMR 2.7 ppm (s, CH_3) and IR (KBr) S=O 1200 cm^{-1} [99].

Reactions for the synthesis of 1-alkyl-5-bromouracils were monitored by TLC, which revealed that bromination is completed before any alkylation occurs. The intermediate **(3)** could possibly be a bromine source in the presence of nucleophilic uracil. In this case the reaction would proceed as shown in the scheme below. Then, the resulting 5-bromouracil would be benzylated after deprotonation with the base.



Example 3

Synthesis of 6-substituted uracils or thiouracils via cyclization



R: Ethyl, Butyl, Phenyl; and X: O or S

In general, to 100 mL of dry ethanol, approximately, 1.1 equivalent of sodium metal was added and the resulting mixture was stirred until the disappearance of sodium particles, which also corresponded to the end of hydrogen evolution. To the resulting solution, 1.0 equivalent of urea or thiourea **(1)** was added and stirred at room temperature until a clear solution occurred. Into this reaction mixture, 1.0 equivalent of β-ketoester **(2)** was added, and the resulting solution was refluxed for two hours or until the product precipitated out solution. Using a rotary evaporator, the solvent was

removed and the obtained slurry was diluted with 75.0 mL of water, cooled in an ice bath and acidified to pH 4.0 with 6N hydrochloric acid. After filtration, the crude uracil product was recrystallized from an appropriate solvent (Table 1), washed with ether, and dried overnight *in vacuo* to afford the pure product **(3)**.

Table 1

Characterization of 6-substituted (thio)uracils.

R	X	Crystalliz. solvent	Yield (%)	Mp (°C) Exp. **(lit.)**	^{1}H NMR (250 MHz) DMSO δ [ppm]	^{13}C NMR DMSO δ [ppm]
Ethyl	S	*i*-propanol	67.6	229-230	12.33 (s, 1H), 12.24 (s, 1H), 5.68 (s, 1H), 2.41 - 2.32 (2H, q), 1.13 - 1.07 (3H, t)	176.0, 161.2, 158.2, 102.1, 24.7, 11.6
Phenyl	S	Ethanol	44.6	260-262 **(263-5)** [100]	12.55 (s, 1H), 12.49 (s, 1H), 7.72 – 7.46 (m, 5H), 6.09 (s, 1H)	176.3, 161.0, 153.0, 131.2, 131.0, 128.7, 127.6, 103.2
Butyl	S	Ethanol	85.3	208-210	12.33 (s, 1H), 12.23 (s, 1H), 5.68 (s, 1H), 2.37 – 2.31 (t, 2H), 1.56 – 1.44 (m, 2H), 1.36 – 1.21 (m, 2H), 0.91 – 0.85 (t, 3H)	176.0, 161.1, 156.9, 102.9, 31.0, 29.2, 21.5, 13.6
Butyl	O	$CHCl_3$-C_6H_{14}	13.5	176-178 **(180.5)** [101]	10.88 (s, 1H), 10.78 (s, 1H), 5.31 (s, 1H), 2.31 – 2.25 (t, 2H), 1.57 – 1.45 (m, 2H), 1.36 – 1.21 (m, 2H), 0.91 – 0.85 (t, 3H)	164.2, 156.6, 151.7, 98.9, 31.4, 29.0, 21.5, 13.6

Example 4

Synthesis of 5-bromo-1,3-dibenzyluracil

To a solution of 5-bromouracil (3.0 g, 15.7 mmol) in dimethylformamide (50.0 mL), potassium carbonate (5.45 g, 39.3 mmol) and benzyl bromide 8.25 g, 47.1 mmol) were added, and the resulting slurry was stirred under argon atmosphere at 50°C for 14 hours. The solvent was removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (100.0 mL) and water (50.0 mL X 2). The aqueous phase was discarded and the organic layer was dried over sodium sulfate. Ethyl acetate was removed on a rotary evaporator, and the residue was purified by flash chromatography (EtOAc/Hexanes 4:1) affording the product 5-bromo-1,3-dibenzyluracil as a white

powder (5.16 g, 88.9%). Mp: 125.5 – 126.5°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 8.51 (s, 1H), 7.39 – 7.22 (m, 10H), 5.02 (s, 2H), 4.96 (s, 2H). ^{13}C NMR: δ 159.67, 151.40, 145.06, 137.46, 137.14, 129.52, 129.22, 128.71, 128.48, 128.18, 95.33, 52.91, 45.91. MS (EI+): 373.3 (10.88%), 372.3 (28.24%), 371.3 (12.55%), 370.2 (30.59%), 279.1 (18.24%), 281.1 (18.63%), 236.0 (22.94%), 238.0 (23.33%), 173.8 (36.47%), 175.9 (34.90%), 91.0 (100%).

Example 5

Synthesis of 6-methyl-1,3-bis-para-nitrobenzyluracil

A mixture of 6-methyluracil (5.0 g, 39.5 mmol), potassium carbonate (5.5 g, 39.8 mmol), dimethylformamide (120.0 mL) and *para*-nitrobenzyl bromide (10.3 g, 47.7 mmol) was stirred under argon atmosphere at 75°C for 9 hours. The solvent was removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (100.0 mL) and water (50.0 mL X 2). The aqueous phase was discarded and the organic layer was dried over sodium sulfate. Ethyl acetate was removed on a rotary evaporator, and the residue was purified by flash chromatography (EtOAc/Hexanes 9:1) affording the product 6-methyl-1,3-*bis-para*-nitrobenzyluracil as a pale yellow powder (5.9 g, 37.7%). No N-monosubstituted product was isolated, and the unreacted starting reagent 6-methyluracil was eluted from column by acetone. Mp: 159 - 161°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.24 – 8.18 (t, J = 8.7 Hz, 4H), 7.54 – 7.48 (t, J = 8.7 Hz), 5.86 (s, 1H), 5.24 (s, 2H), 5.12 (s, 2H), 2.18 (s, 3H). ^{13}C NMR: δ 162.10, 154.14, 152.90, 147.65, 147.51, 145.80, 145.50, 129.23, 128.10, 124.80, 124.46, 101.80, 48.03, 44.31, 20.12. MS (EI+): 397.3 (1.2%), 396.3 (5.3%), 261.2 (3.4%), 217.1 (9.4%), 136.0 (14.1%), 110.0 (100%).

Example 6

Reaction of 6-methyluracil and p-nitrobenzyl bromide

To a suspension of 6-methyluracil (4.2 g, 33.0 mmol) in dry DMF (40.0 mL) 1,8-diazabicyclo[5,4,0]-undec-7-ene or DBU (5.9 mL, 39.0 mmol) was slowly added resulting in the formation of a clear solution. This solution was stirred at room

temperature for 3 hours, then *para*-nitrobenzyl bromide (7.2 g, 33 mmol) was added, and the resulting mixture was stirred for four hours at 60°C. The solvent was removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (100.0 mL) and water (50.0 mL X 2). The organic phase was dried over magnesium sulfate, the ethyl acetate was removed on a rotary evaporator, and the resulting residue was crystallized from acetone affording the product 6-methyl-3-*para*-nitrobenzyluracil as a white powder (1.9 g, 20.9%). Mp: > 245°C (decomposes to a brown solid that melts between 265 – 280°C). The position of the *para*-nitrobenzyl group was confirmed by a NOESY experiment that showed a strong correlation between the N_1 proton and methyl protons, while no correlation was observed between the *para*-nitrobenzyl CH_2 and the methyl protons. ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.30 (s, 1H), 8.19 – 8.16 (d, J = 8.7 Hz, 2H), 7.51 – 7.48 (d, J = 8.7 Hz, 2H), 5.56 (s, 1H), 5.04 (s, 2H), 2.07 (s, 3H). ^{13}C NMR: δ 153.52, 152.92, 152.26, 147.44, 146.16, 129.23, 124.41, 99.10, 42.98, 19.03. MS (EI+): 261.1 (14.0%), 212.2 (10.68%), 211.1 (77.3%), 183.9 (23.2%), 156.0 (65.3%), 151.7 (20.2%), 141.9 (18.0%), 127.8 (89.3%), 112.1 (13.5%), 109.8 (32.8%), 84.5 (100%).

All filtrates from 6-methyl-3-*para*-nitrobenzyluracil crystallization were collected, and purification by flash chromatography produced 3.6 g or 27.9% yield of 6-methyl-1,3-*bis*-*para*-nitrobenzyluracil.

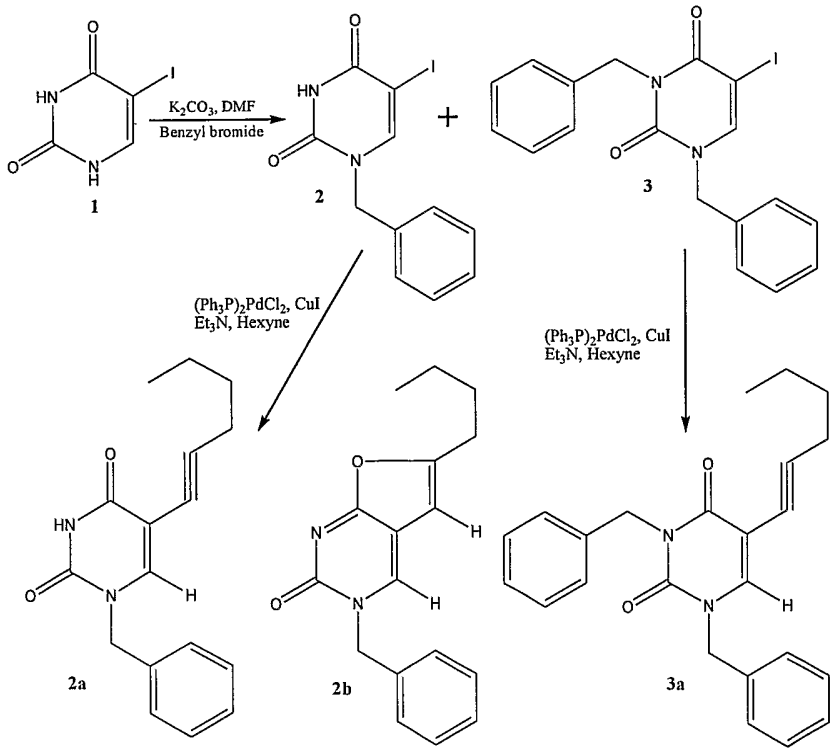
Example 7

Bromination of 6-methyl-3-para-nitrobenzyluracil

Bromine (0.3 mL, density 3.119, 5.8 mmol) was added to a solution of 6-methyl-3-*para*-nitrobenzyluracil (1.2 g, 4.6 mmol) in DMF (20.0 mL), and the reaction mixture was stirred at 65°C for 4 hours. The solvent was removed by vacuum distillation and the resulting residue was stirred in dichloromethane (100.0 mL) precipitating the product as a white solid. After filtration, washing with dichloromethane, and drying overnight under vacuum, the compound 5-bromo-6-methyl-3-*para*-nitrobenzyluracil was obtained as a white powder in quantitative yield. Mp 225 – 227°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.80 (s, 1H), 8.20 – 8.17 (d, J = 8.4 Hz, 2H), 7.54 – 7.51 (d, J = 8.4 Hz, 2H), 5.10 (s, 2H), 2.26 (s, 3H). ^{13}C NMR: δ 159.99, 151.61, 151.04, 147.53, 145.58, 129.34, 124.43, 95.36, 44.61, 35.13.

Example 8

A. Preparation of 1-benzyl-5-iodouracil (2) and 1,3-dibenzyl-5-iodouracil (3)



A mixture of 5-iodouracil (4.6 g, 19.3 mmol), potassium carbonate (2.7 g, 19.3 mmol) and dry DMF (40 mL) was stirred under nitrogen atmosphere at 45°C for 30 minutes resulting into a white slurry. Benzyl bromide (2.3 mL, 19.3 mmol) was added and the reaction mixture was stirred to give a pale yellow solution and insoluble potassium carbonate as a white precipitate. After three hours, an extra 2.3 mL of benzyl bromide was added, and the reaction proceeded for three more hours until the disappearance of the starting 5-iodouracil as observed by TLC. The solvent was completely removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (200.0 mL) and water (80 mL X 2). The aqueous phase was discarded and the organic layer was dried over magnesium sulfate. Ethyl acetate was removed on a rotary evaporator, and the residue was crystallized from chloroform. After filtration, washing with cold chloroform and drying overnight *in vacuo* the product **(2)** was obtained as a white powder (2.3 g, 36.3%). Mp: 217 - 219°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.72 (s, 1H), 8.35 (s, 1H), 7.39 – 7.28 (m, 5H), 4.88 (s, 2H). ^{13}C NMR: δ 161.90, 151.60, 150.63, 137.55, 129.52, 128.61, 128.36, 69.52, 51.35. MS (EI+): 329.2 (9.6%), 328.0 (100%), 165.7 (38.1%), 131.8 (40.9%), 126.8 (49.3%), 101.7 (6.9%).

The chloroform filtrate and washings were collected and purified by flash chromatography to afford the compound **(3)** as a white powder (3.6 g, 44.6%). Mp 109-111°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.48 (s, 1H), 7.36 – 7.26 (m, 10H), 5.02 (s, 2H), 4.95 (s, 2H). ^{13}C NMR: δ 160.79, 151.77, 149.53, 137.61, 137.32, 129.52, 129.21, 128.68, 128.49, 128.41, 128.14, 68.60, 52.71, 46.01. MS (EI): 419.1 (23.9%), 418.0 (70.1%), 328.1 (3.4%), 327.0 (38.5%), 283.9 (41.0%), 221.8 (48.9%), 131.8 (100%), 127 .7 (17.9%), 104.1 (28.2%).

B. Synthesis of 1-benzyl-5-(hex-1-ynyl)pyrimidine-2,4(1H,3H)-dione (2a) and 3-benzyl-6-(n-butyl)furo[2,3-d]pyrimidine-2,(3H)-one (2b)

A suspension of 1-benzyl-5-iodouracil (1.3 g, 4.0 mmol) in dry triethylamine (70.0 mL) was vigorously stirred and deoxygenated by bubbling nitrogen through the resulting white slurry. Cuprous iodide (27.3 mg), [bis-(triphenylphosphine)]-palladium chloride (26.3 mg) and 1-hexyne (1.0 mL, 8.7 mmol) were added and the reaction

mixture was stirred at 50°C for 2 hours. The solvent was removed on a rotary evaporator, and the resulting residue was dissolved in 100.0 mL of chloroform, extracted twice with 50.0 mL of 5% aqueous solution of the sodium salt of ethylene-diamine-tetra-acetic acid (EDTA), and once with 50.0 mL of water. The blue aqueous phase was discarded and the brown organic layer was dried over sodium sulfate, treated with activated Norit charcoal and filtered through celite to give a bright yellow solution. After purification by flash chromatography (Ether/$CHCl_3$ 4:1, then 100% $CHCl_3$), compounds **(2a)** and **(2b)** were obtained as white and pale yellow solids, respectively. Isolated in 26.0% yield (293.7 mg), the product **(2a)** had an rf value of 0.68 (Hexanes/EtOAc 1:2) and mp: 158 - 159°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.60 (s, 1H), 8.11 (s, 1H), 7.36 – 7.30 (m, 5H), 4.87 (s, 2H), 2.38 – 2.34 (t, J = 6.9 Hz, 2H), 1.46 – 1.38 (m, 4H), 0.90 – 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR: δ 163.12, 150.92, 148.68, 137.45, 129.52, 128.61, 128.41, 99.46, 94.16, 73.46, 51.41, 31.09, 22.23, 19.30, 14.34. MS (EI+): 283.4 (0.9%), 282.1 (5.9%), 267.1 (3.5%), 191.0 (15.4%), 148.7 (10.1%), 91.2 (100%), 64.9 (64.3%).

The product **(2b)**, fluorescent under ultraviolet light, was obtained in 11.0% yield (122.4 mg), and had an rf value of 0.21 (Hexanes/EtOAc 1:2). Mp 198 - 199°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.60 (s, 1H), 7.35 – 7.29 (m, 5H), 6.44 (s, 1H), 5.14 (s, 2H), 2.66 – 2.62 (t, J = 7.2 Hz, 2H), 1.62 – 1.57 (m, 2H), 1.38 – 1.31 (m, 2H), 0.92 – 0.88 (t, J = 7.2 Hz, 3H) ^{13}C NMR: δ 172.28, 159.20, 155.41, 142.67, 137.66, 129.44, 128.60, 107.53, 100.34, 54.19, 29.29, 27.90, 22.37, 14.44. MS (EI+): 283.3 (2.3%), 282.2 (9.2%), 281.0 (1.8%), 239.1 (1.5%), 190.9 (3.1%), 176.0 (4.2%), 91.1 (100%), 64.9 (26.6%).

C. Synthesis of 1,3-Dibenzyl-5-(hex-1-ynyl)pyrimidine-2,4(1H,3H)-dione (3a)

Following the same synthetic procedure as for **(2a)**, the compound **(3a)**, was prepared from 1,3-dibenzyl-5-iodouracil (1.8 g, 4.3 mmol), cuprous iodide (25.0 mg), [bis-(triphenylphosphine)]-palladium chloride (26.3 mg) and 1-hexyne (1.0 mL, 8.7 mmol) at 40°C for two hours. After discoloration of the organic phase ($CHCl_3$) with activated Norit charcoal, and purification by flash chromatography ($CHCl_3$), the compound **(3a)** was obtained as a fine powder (827.2 mg, 51.7%). Like **(2a)**, the

product **(3a)** was also non-fluorescent under UV light. It had an R_f value of 0.82 (Hexanes/EtOAc 1:2), mp: 99 - 100°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.24 (s, 1H), 7.37 – 7.23 (m, 10H), 4.98 (s, 2H), 4.95 (s, 2H), 2.40 – 2.35 (t, J = 6.6 Hz, 2H), 1.48 – 1.38 (m, 4H), 0.91 – 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR: δ 162.14, 151.17, 147.48, 137.63, 137.22, 129.52, 129.20, 128.68, 128.42, 128.10, 98.79, 94.21, 73.51, 52.77, 45.02, 31.05, 22.20, 19.27, 14.33. MS (EI+): 372.2 (1.2%), 330.1 (0.6%), 281.1 (5.7%), 253.0 (0.4%), 238.6 (5.7%), 131.9 (3.7%), 91.0 (100%), 76.8 (5.5%), 64.9 (12.5%).

Example 9

Iodination of 6-methyl-1,3-bis-para-nitrobenzyluracil

Iodomonochloride (0.7 g, 4.3 mmol) was added to an ice pre-cooled yellow solution of 6-methyl-1,3-*bis-para*-nitrobenzyluracil (1.0 g, 2.5 mmol) in dichloromethane (15.0 mL) resulting in the formation of a dark red solution. This solution was stirred at 0°C for 2 hours, then at 45°C for 16 hours in an attempt to drive the reaction to completion. After purification by flash chromatography (EtOAc/Hexanes 3:5), and drying overnight under vacuum, the product 5-iodo-6-methyl-1,3-*bis-para*-nitrobenzyluracil was obtained as a white solid (419.6 mg, 32.2%). Mp: 218 - 220°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.23 – 8.18 (m, 4H), 7.57 – 7.53 (m, 4H), 5.40 (s, 2H), 5.20 (s, 2H), 2.52 (s, 3H). ^{13}C NMR: δ 160.27, 154.88, 152.36, 147.68, 147.58, 145.52, 145.24, 129.39, 128.14, 124.77, 124.44, 76.24, 50.34, 46.35, 26.20. MS (EI+): 522.09 (0.9%), 361.23 (1.34%), 287.20 (1.47%), 231.11 (3.1%), 166.71 (6.4%), 148.86 (20.6%), 116.75 (22.2%), 106.00 (28.2%), 104.44 (55.9%), 77.43 (73.4%), 68.86 (90.1%), 54.90 (100%).

Example 10

Synthesis of 5-bromo-1-para-nitrobenzyluracil

A milky mixture of 5-bromouracil (2.0 g, 10.5 mmol), chlorotrimethylsilane (2.5g, 23.1 mmol) and 50.0 mL of 1,1,1,3,3,3-hexamethyldisilizane (HMDS) was stirred under an argon atmosphere at 125°C until a clear solution was formed. After cooling down to 60°C, *para*-nitrobenzyl bromide (2.3g, 10.6 mmol) was added and the reaction

mixture was stirred for 40 hours. The solvent was removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (100.0 mL) and water (50.0 mL X 2). The aqueous phase was discarded and the organic layer was dried over sodium sulfate. Ethyl acetate was removed on a rotary evaporator, and the residue was purified by flash chromatography (100% chloroform) affording the product 5-bromo-1-*para*-nitrobenzyluracil as pale yellow powder (836.9 mg, 24.4%). Mp: 258 - 260°C (decomposes). ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.90 (s, 1H), 8.42 (s, 1H), 8.23 – 8.21 (d, J = 8.4 Hz, 2H), 7.60 – 7.57 (d, J = 8.7 Hz, 2H), 5.02 (s, 2H). ^{13}C NMR: (100 MHz, DMSO d_6): δ 160.43, 151.16, 147.73, 145.95, 144.96, 129.29, 124.48, 124.32, 96.26, 51.20. MS (EI): 328.2 (1.5%), 327.1 (11.8%), 326.2 (2.1%), 325.1 (12.0%), 175.8 (4.0%), 136.0 (100%), 119.8 (14.6%), 105.8 (61.2%), 89.0 (64.6%), 77.8 (93.9%), 62.8 (32.6%).

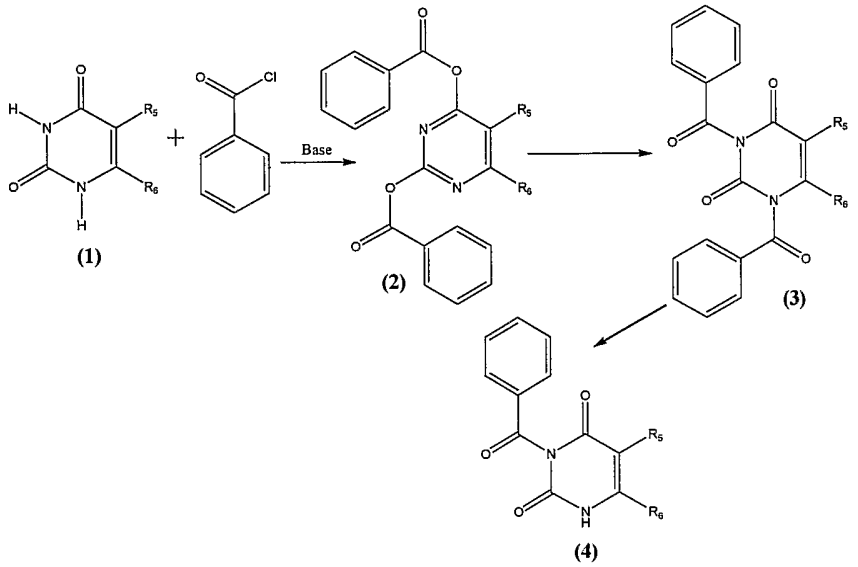
Example 11

Bromination of 6-methyluracil

Pre-cooled liquid bromine (4.1 mL, density 3.1) was slowly added to an ice cold suspension of 6-methyluracil in aqueous sodium hydroxide solution (1.0 M, 80.0 mL), and the resulting mixture was vigorously stirred in an ice bath for 1 hour, and then allowed to warm to room temperature. In an attempt to drive the reaction to completion, the reaction mixture was heated to 50°C, and bromine was slowly added until a persistent yellow color was obtained. The pale yellow reaction mixture was refluxed until the yellow color disappeared, resulting in a white slurry. The reaction mixture was cooled in ice bath; filtered; washed with water, ethanol and ether; and dried under vacuum overnight to afford the compound 5-bromo-6-methyluracil in quantitative yield.

Example 12

Benzoylation Reactions



In this reaction, without wishing to be bound by theory, in certain embodiments there appears to be competition between O- and N- benzoylation leading to the kinetic product **(2)** and thermodynamic product **(3)**, respectively. When heated, the compound **(2)** readily converts to **(3)**, which may easily undergo N_1 deprotection affording **(4)**, especially when R_6 is a proton.

A. Benzoylation of uracil and analogues

Under a nitrogen atmosphere, benzoyl chloride (25.0 mL, 218.5 mmol) was slowly added to a mixture of uracil (10 g, 87.4 mmol) and pyridine (18.0 mL, 218.5 mmol) in dry acetonitrile (30.0 mL), resulting in the formation of a bright yellow solution after stirring for ten minutes. The reaction mixture was then stirred overnight (14 hours) at room temperature precipitating the product as a pale yellow solid. The solvent was removed by vacuum distillation and the resulting slurry was extracted with ethyl acetate and water. The organic layer was dried over sodium sulfate and the solvent removed on rotary evaporator to afford a yellowish crude product. After crystallization from boiling ethanol and drying overnight under vacuum, the compound 3-benzoyluracil was obtained as white powder (16.9g, 89.5%). Mp: >203°C (decomposes). ^{1}H NMR:

(250 MHz, DMSO d_6): δ 11.63 (broad s, 1H), 7.97 – 7.57 (m, 6H), 5.76 – 5.73 (d, J = 7.6 Hz, 1H). ^{13}C NMR: δ 170.04, 162.95, 150.07, 143.32, 135.43, 131.34, 130.23, 129.52, 100.11. MS (EI+): HRMS (Experimental): 216.0517±0.0008; calculated: 216.0535

5

B. Synthesis of 3-benzoyl-5-bromo-1-para-nitrobenzyluracil

With the exception that triethylamine (4.0 mL) was used as base instead of pyridine, this compound was prepared according to the benzoylation procedure starting from 5-bromo-1-*para*-nitrobenzyluracil (1.5 g, 4.6 mmol) and benzoyl chloride (1.0 mL, 10 8.7 mmol). After 14 hours of reaction time at room temperature, crystallization from ethyl acetate and recrystallization from ethyl acetate - ether, the compound 3-benzoyl-5-bromo-1-*para*-nitrobenzyluracil was obtained as needle-like crystals (1.7g, 87.4%). Mp: 184 - 186°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.69 (s, 1H), 8.26 – 8.25 (d, J = 3.6 Hz, 2H), 8.23 – 8.22 (d, J = 2.1 Hz, 2H), 8.07 – 7.58 (m, 5H), 5.11 (s, 2H). ^{13}C NMR: δ 15 169.29, 149.95, 147.95, 147.06, 144.39, 136.69, 131.50, 130.42, 129.53, 124.61, 95.79, 52.11.

C. Benzoylation of 5-bromo-6-methyluracil

Following the benzoylation procedure, this reaction failed to proceed at room 20 temperature. However, under reflux, the reaction between 5-bromo-6-methyluracil (9.6 g, 46.8 mmol), benzoyl chloride (11.9 mL, 103.5 mmol), and pyridine (8.5 mL, 103.2 mmol) in acetonitrile (100.0 mL) was completed within 7 hours. After ethyl acetate / water extraction and the usual work-up of the organic phase, crystallization of the solid crude product from methanol afforded the compound 1,3-dibenzoyl-5-bromo-6-25 methyluracil as white powder (4.5g, 23.3%). Mp: 184 – 184.5°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.26 (m, 4H), 7.86 – 7.79 (m, 2H), 7.68 – 7.60 (4H), 2.33 (s, 3H). ^{13}C NMR: δ 169.14, 168.54, 158.53, 149.74, 148.78, 137.17, 136.86, 131.87, 131.66, 131.38, 130.58, 130.43, 100.29, 20.69. MS (EI+): 414.1 (0.3%), 412.1 (0.3%), 333.2 (0.4%), 308.0 (0.3%), 282.0 (1.9%), 280.0 (2.1%), 229.0 (9.0%), 162.8 (1.7%), 160.8 30 (1.8%), 121.8 (15.3%), 107.3 (35.9%), 105.1 (100%).

The methanol filtrate was evaporated to dryness and the resulting solid was recrystallized from acetonitrile to afford the compound 3-benzoyl-5-bromo-6-methyluracil as white powder (5.0 g, 33.3%). Mp: 210 – 212°C (decomposes). ^{1}H NMR: (300 MHz, DMSO d_6): δ 12.06 (s, 1H), 8.03 – 8.00 (d, J = 8.5 Hz, 2H), 7.82 – 7.77 (m, 1H), 7.64 – 7.58 (t, J = 7.7 Hz, 2H), 2.32 (s, 3H). ^{13}C NMR: δ 169.87, 159.48, 153.74, 149.63, 136.48, 131.83, 131.34, 130.37, 95.23, 20.75. MS (EI+): 311.0 (1.2%), 309.0 (1.2%), 282.0 (3.2%), 280.0 (3.5%), 229.0 (9.5%), 118.9 (18.7%), 116.9 (21.5%), 105.1 (100%).

Example 13

Alkylation of benzoyl-protected uracil analogs

A. 3-benzoyl-1-para-nitrobenzyluracil (Alkylation procedure)

To an ice bath pre-cooled mixture of sodium hydride (0.6 g, 25.0 mmol) and dry DMF (25.0 mL) was slowly added a yellow solution of 3-benzoyluracil (4.86 g, 22.5 mmol) in DMF (25.0 mL). After vigorous stirring for half an hour (or until hydrogen evolution stopped), a pre-cooled yellow solution of *para*-nitrobenzyl bromide (5.4g, 25.0 mmol) in DMF (25.0 mL) was added drop-wise to the reaction mixture resulting in a red solution. The solution thus obtained was stirred in an ice bath for an additional hour, then overnight at room temperature. The solvent was removed by vacuum distillation and the resulting residue was extracted with ethyl acetate (200.0 mL) and water (75.0 mL X 2). The aqueous phase was discarded and the organic layer was dried over magnesium sulfate. Ethyl acetate was removed on a rotary evaporator and the resulting red solid was crystallized from acetonitrile, washed with diethyl ether, and dried overnight under vacuum to afford the compound 3-benzoyl-1-*para*-nitrobenzyluracil as pale yellow powder (6.8 g, 85.9%). Mp: 168 - 170°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 8.27 – 8.23 (m, 2H), 8.10 – 8.08 (d, J = 8.1 Hz, 1H), 7.99 – 7.97 (m, 2H), 7.81 – 7.76 (m, 1H), 7.64 – 7.58 (4H), 5.98 – 5.96 (d, J = 8.1 Hz, 1H), 5.12 (s, 2H). ^{13}C NMR: δ 170.31,163.13, 150.48, 147.92, 147.56, 144.78, 136.44, 131.86, 131.18, 130.40, 129.40, 124.71, 102.26, 51.62. MS (EI+): 351.2 (0.5%), 325.3 (2.4%), 324.3 (18.8%), 323.2 (75.6%), 282.2 (0.9%), 281.2 (5.42%), 204.0 (14.4%), 156.9 (9.2%), 135.9 (13.1%), 129.0 (11.1%), 106.4 (100%), 104.8 (100%).

B. 3-Benzoyl-1- benzyl-5-iodouracil

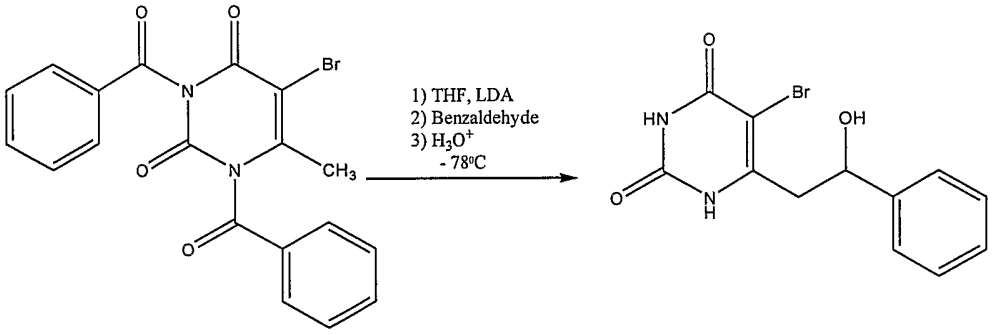
Following the procedure for the alkylation of benzoyl-protected uracils, this compound was prepared from the reaction of sodium hydride (0.4 g, 16.4 mmol), 3-benzoyl-5-iodouracil (5.1 g, 14.9 mmol), and benzyl bromide (22.3 mmol) for a total time of 14 hours. After the typical work-up, the product 3-benzoyl-1-benzyl-5-iodouracil was obtained as white powder (4.4 g, 68.0%). Mp: 194 - 196°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 8.61 (s, 1H), 8.01 – 7.30 (m, 10H), 4.96 (s, 2H). ^{13}C NMR: δ 168.87, 159.69, 150.64, 149.46, 136.06, 135.71, 130.69, 130.53, 129.57, 128.77, 127.98, 127.67, 67.84, 51.37. MS (EI+): HRMS (Experimental): 431.9953±0.0008; calculated: 431.9971 amu.

C. 3-Benzoyl-1-benzyluracil

This compound was prepared following the procedure for alkylation of benzoyl-protected uracils from the reaction of 3-benzoyluracil (4.1 g, 19.0 mmol), sodium hydride (504 mg, 21.0 mmol), and benzyl bromide (21.0 mmol) for a total time of 12 hours. After ethyl acetate-water extraction, the aqueous phase was discarded and the organic layer was dried over sodium sulfate. The residue from the organic phase was purified by column chromatography (ethyl acetate / hexanes: 2:3) affording the product 3-benzoyl-1-benzyluracil as white powder (4.8 g, 82.1%). Mp: 107 - 108°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 8.61 (s, 1H), 8.01 – 7.30 (m, 10H), 4.96 (s, 2H). ^{13}C NMR: δ 168.87, 159.69, 150.64, 149.46, 136.06, 135.71, 130.69, 130.53, 129.57, 128.77, 127.98, 127.67, 67.84, 51.37. MS (EI+): 306.1 (4.8%), 278.1 (2.9%), 158.2 (4.5%), 130.2 (2.2%), 105.0 (100%), 91.1 (28.4%), 77.0 (63.2%), 65.0 (12.1%), 51.0 (15.2%). High resolution MS: experimental value: 306.1009 ± 0.0008 amu, calculated: 306.1004 amu.

Example 14

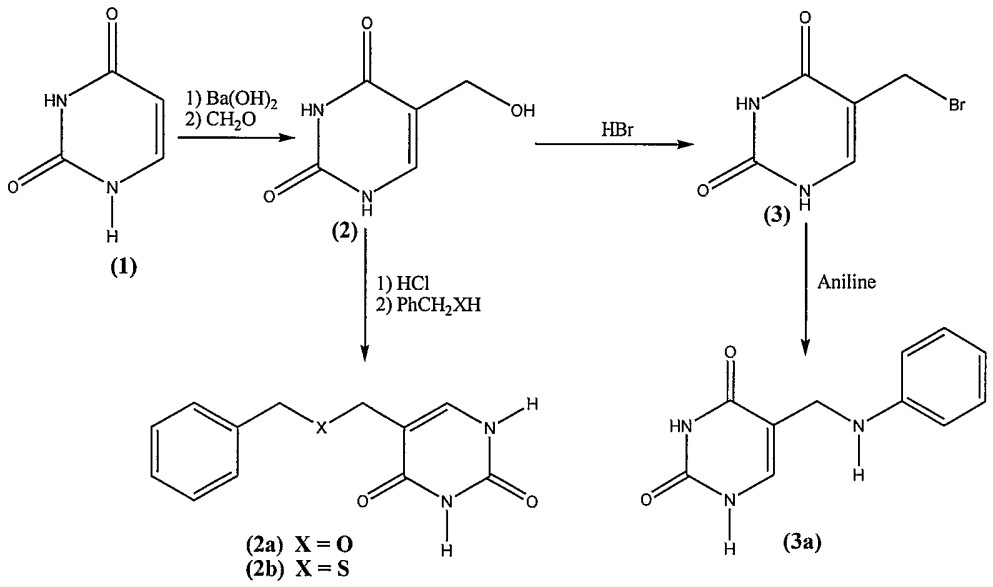
5-bromo-6-(2-hydroxy-2-phenylethyl)uracil



To a pre-cooled solution of 1,3-dibenzoyl-5-bromo-6-methyluracil (1.0 g, 2.4 mmol) in THF (25.0 mL) was slowly added LDA solution in THF/Heptane (1.5 mL, 2.9 mmol) under an argon atmosphere. After fifteen minutes of stirring, benzaldehyde (0.33 mL, 2.9 mmol) was added, and the reaction mixture was stirred for an additional hour at –78°C, then allowed to warm to room temperature and acidified with hydrochloric acid. After ethyl acetate – water extraction, the aqueous phase was discarded and the organic layer was dried over sodium sulfate. Ethyl acetate was removed on a rotary evaporator and the resulting residue was crystallized from chloroform – ether to give the product 5-bromo-6-(2-hydroxy-2-phenylethyl)uracil as pale yellow powder (447.5 mg, 59.6%). Mp >205°C (decomposes). ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.46 (s, 1H), 11.23 (s, 1H), 7.44 – 7.26 (m, 5H), 5.64 – 5.63 (d, J = 4.2 Hz, 1H), 5.02 – 4.99 (broad t, J = 4.5 Hz, 1H), 2.92 – 2.76 (m, 2H). ^{13}C NMR: δ 161.06, 152.74, 151.14, 145.26, 129.03, 128.12, 126.38, 97.11, 70.81, 43.73. MS (CI+): 330.3 (M + NH_4^+), 312.2 ($M+H^+$), 311.2 (M^+), 295.2 (5.9%), 293.1 (5.3%), 231.1 (7.6%), 223.9 (13.6%), 215.0 (33.24), 213.0 (16.8%), 144.0 (40.6%), 127.0 (100%), 121.9 (8.6%), 110.8 (9.6%).

Example 15

A. 5-(hydroxymethyl)uracil (2) and 5-(bromomethyl)uracil (3)



Compounds **(2)** and **(3)** were prepared in 75.8% and 85.6% yield respectively following the literature procedure by Knochel et al. [102]. Compound **(2)** was obtained from the reaction of uracil and formaldehyde (37% aqueous solution) in the presence of barium hydroxide. Compound **(3)** was prepared by refluxing compound **(2)** with concentrated HBr.

B. 5-(benzyloxymethyl)uracil (2a)

A suspension of 5-(hydroxymethyl)uracil (2.5 g, 17.6 mmol), concentrated aqueous hydrochloric acid (1.5 mL), and benzyl alcohol (63.0 mL) was gently refluxed for one hour, resulting in the formation of a pale yellow solution. The reaction mixture was then cooled to room temperature and ether (330.0 mL) was added while stirring. The resulting fine precipitate was filtered, washed with ether and dried overnight under vacuum to afford the pure product **(2a)** as white powder (3.2 g, 79.2%). Mp 194 - 196°C (lit. mp 199 – 202°C [102]; mp 202 - 203°C [103]). ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.14 (s, 1H), 10.86 (broad s, 1H), 7.45 – 7.43 (d, J = 3.4 Hz, 1H), 7.32 – 7.24 (m, 5H),

4.48 (s, 2H), 4.15 (s, 2H). ^{13}C NMR: δ 163.86, 151.34, 140.77, 138.50, 128.23, 127.47, 127.38, 109.07, 71.34, 64.24.

C. 5-(benzylthiomethyl)uracil (2b)

A suspension of 5-(hydroxymethyl)uracil (2.1 g, 14.8 mmol), concentrated aqueous hydrochloric acid (4.0 mL), and benzylmercaptan (3.4 mL) in THF (50.0 mL) was gently refluxed for 12 hours, resulting in the formation of a clear solution. The reaction mixture was then cooled to room temperature and ether (100.0 mL) was added while stirring. The resulting fine precipitate was filtered, washed with ether and dried overnight under vacuum to afford the pure product **(2b)** as white powder (2.1 g, 57.4%). Mp 214 - 215°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.13 (s, 1H), 10.78 – 10.76 (d, J = 4.9 Hz, 1H), 7.32 – 7.22 (m, 6H), 3.72 (s, 2H), 3.28 (s, 2H). ^{13}C NMR: δ 163.77, 151.20, 139.16, 138.57, 128.87, 128.37, 126.77, 109.30, 35.61, 27.16. High resolution MS: experimental value: 248.0615 ± 0.0008 amu, calculated value: 248.0619 amu.

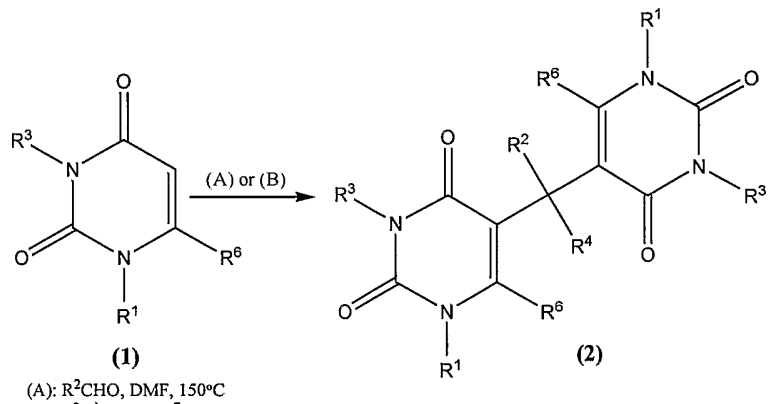
D. 5-(phenylaminomethyl)uracil (3a)

A suspension of 5-(bromomethyl)uracil (1.0 g, 4.9 mmol), aniline (10.0 mL) in 50% by volume THF/DMSO (20.0 mL) was gently refluxed for 16 hours, resulting in the formation of a clear solution. The solvents were then removed by vacuum distillation and the residual slurry was diluted with chloroform (40.0 mL) and ether (20.0 mL). The mixture was then stirred and the resulting fine precipitate was filtered and washed with ether. Recrystallization from ethanol afforded compound **(3a)** in 77.4% yield (819.4 mg). Mp 238 - 240°C (decomposes). ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.15 (s, 1H), 10.73 – 10.71 (d, J = Hz, 1H), 7.24 – 7.22 (d, J = Hz, 1H), 7.09 – 7.03 (m, 2H), 6.57 – 6.50 (m, 3H), 5.76 (broad s, 1H), 3.83 (s, 2H). ^{13}C NMR: δ 164.28, 151.29, 148.26, 138.58, 128.93, 116.00, 112.27, 109.60, 38.82. High resolution MS: experimental value: 217.0851 ± 0.0008, calculated: 217.0851 amu.

Example 16

Reactions of Uracils

The present invention also includes two efficient processes for the conversion of uracil derivatives **(1)** into their corresponding 5,5'-methylene-*bis*-uracils **(2)** using readily available aldehydes or sulfoxides.



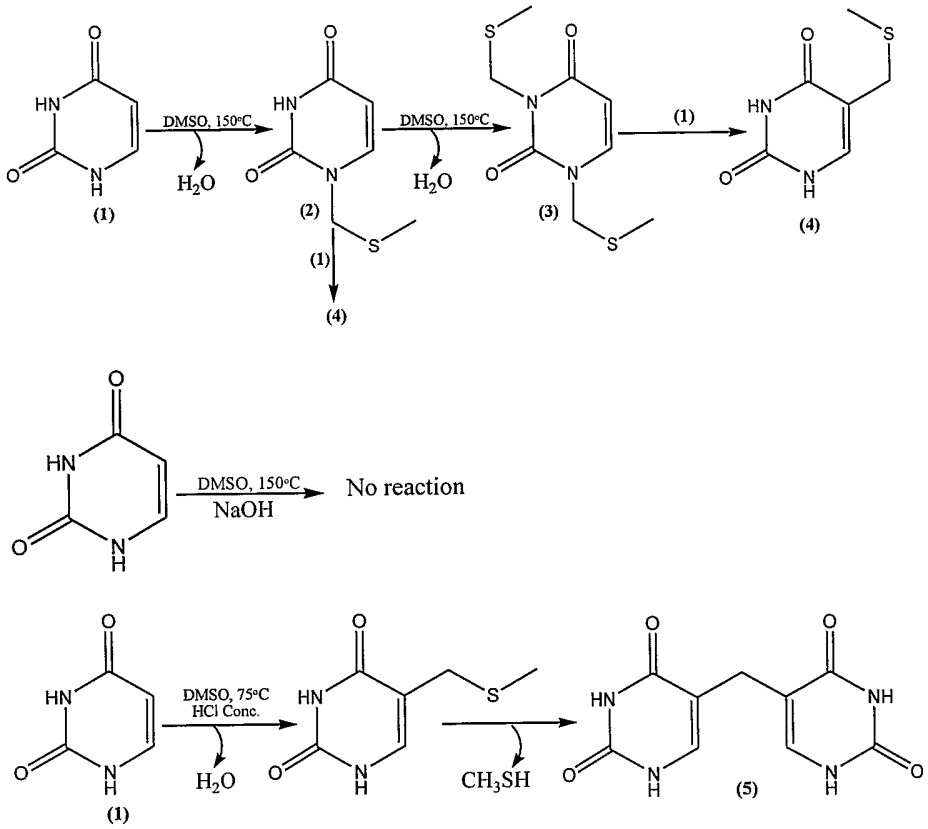
(1) **(2)**

(A): R^2CHO, DMF, 150°C
(B): $R^2R^4CHS(O)R^7$, conc. HCl, 75°C

Under reaction conditions (A), the product **(2)** is readily obtained from an alcohol intermediate upon dehydration while conditions (B) yield the same product from a sulfide intermediate following a loss of the mercaptan HSR7 *via* elimination or nucleophilic substitution.

Uracil reacts slowly with dimethyl sulfoxide at high temperatures (>125°C) to yield N and C-5 substituted products in low yields under neutral conditions, in addition to trace amounts of 5,5'-methylene-bis-uracil. Although in certain embodiments, there is no reaction observed under basic conditions, both reaction yields and times are significantly improved when reactions are carried out under acidic conditions. Acetic acid, toluene-para-sulfonic acid and hydrochloric acid were tried in these reactions, but the latter appears to offer the best results in terms of reaction times, yields and ease of purification. Furthermore, hydrochloric acid catalyzed reactions do not require very high temperatures, as they very often proceed well at 75°C. Under these conditions, no N-substituted uracil derivatives were observed, and 5,5'-methylene-bis-uracil became the

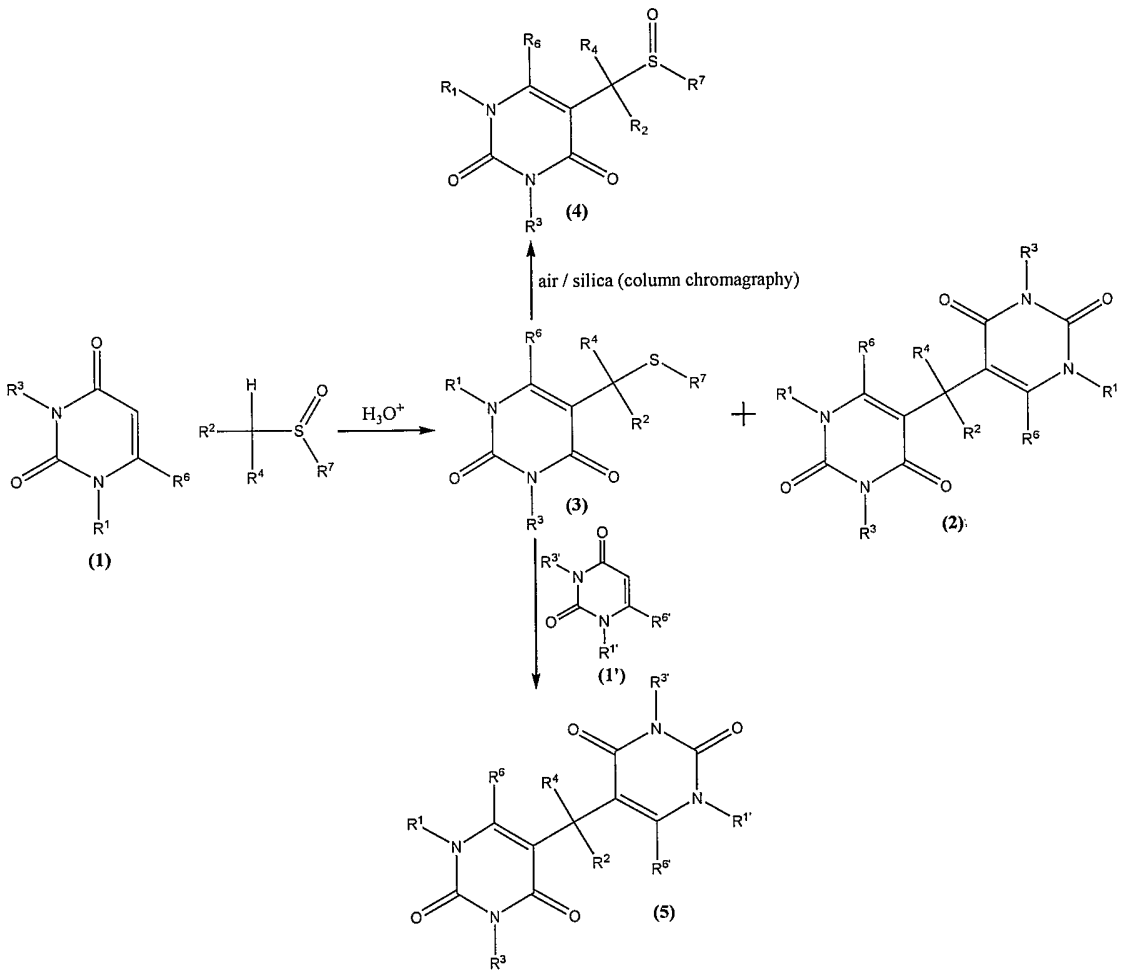
major product with the sulfide or sulfoxide as minor products. Several examples of reactions that were examined during the analysis are depicted below.



Under neutral conditions, all the five products are observed in ratios that vary with reaction time and, possibly the dryness level of DMSO. Acidic or dryer conditions favor **(5)**, otherwise **(3)** is the major product even though, the overall uracil conversion to products is low. However, under acidic conditions, no N-substituted products were detected in the course of the reaction. Without wishing to be bound by theory, this may be due to reduced or non-existent nucleophilicity of the ring N atoms as uracil primarily adopts its 2,4-dihydroxypyrimidine structure.

I Acid-catalyzed reactions of sulfoxides and uracils

The general reaction scheme for acid-catalyzed reactions of sulfoxides and uracils may be characterized by the following reaction scheme:

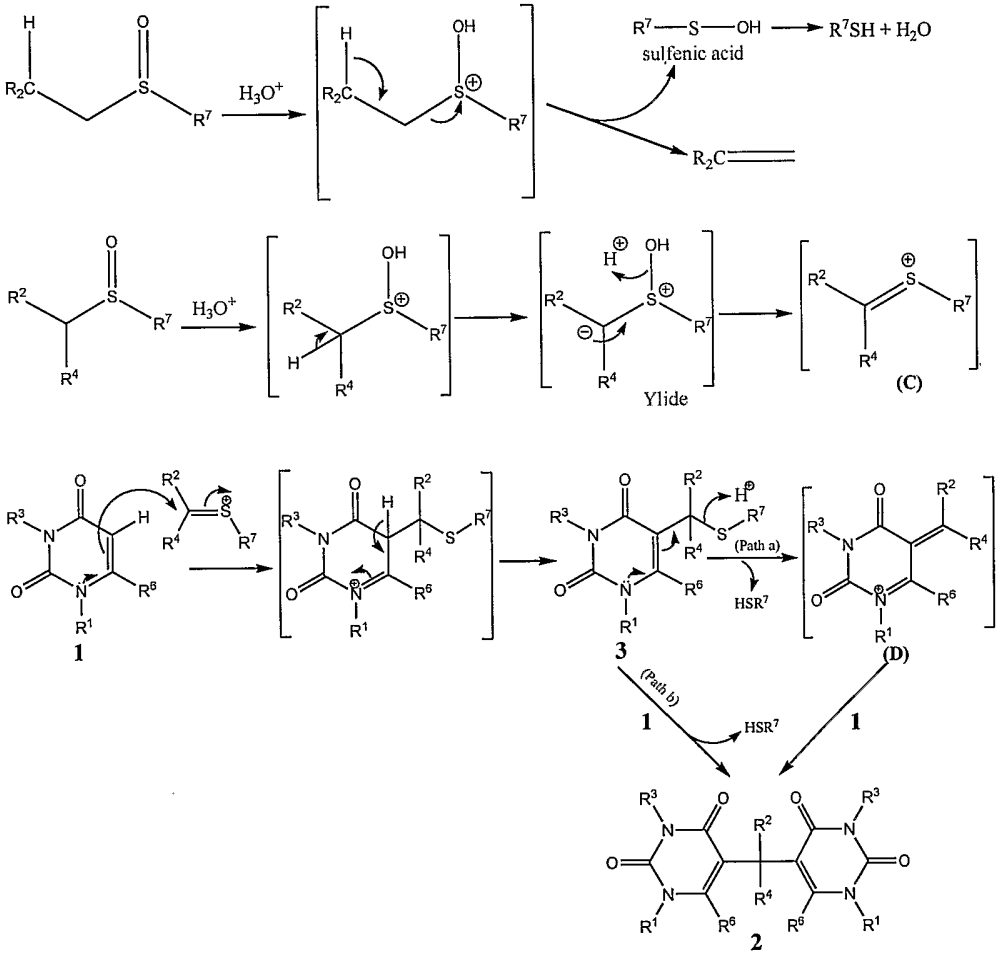


Several examples of compounds prepared under this reaction scheme are shown in Table 1A below.

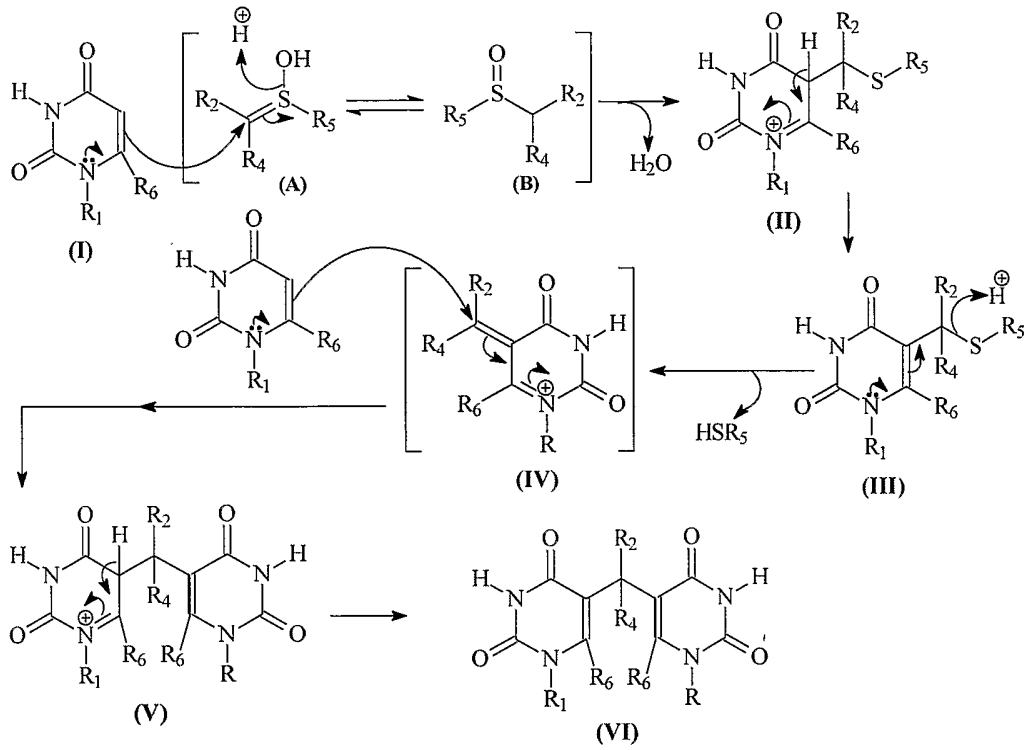
Table 1A: Products from acid-catalyzed reactions of uracils & sulfoxides

R^3=H	R^1	R^6	R^2	R^4	R^7	Sulfide **3** % Yield / Mp (°C)	Sulfoxide **4** % Yield / Mp (°C)	Derivative **2** % Yield / Mp (°C)
(b)	H	H	H	H	CH_3	10.1 / 236-9	1.9 / 228-30	57.8 / >350 dec.
(c)	H	CH_3	H	H	CH_3	0 / NA	0 / NA	89.7 / >300 dec.
(d)	H	H	Phenyl	H	Benzyl	0 / NA	1.5 / 253-5	60.0 / 318-23 dec.
(e)	H	CH_3	Phenyl	H	Benzyl	0 / NA	0 / NA	51.2 / 352-4
(f)	Benzyl	H	Phenyl	H	Benzyl	0 / NA	0 / NA	48.9 / 256-8
(g)	H	H	n-Propyl	H	n-Butyl	0 / NA	0 / NA	0 / NA
(h)	H	H	Phenyl	Phenyl	Benzyl	0 / NA	0 / NA	0 / NA

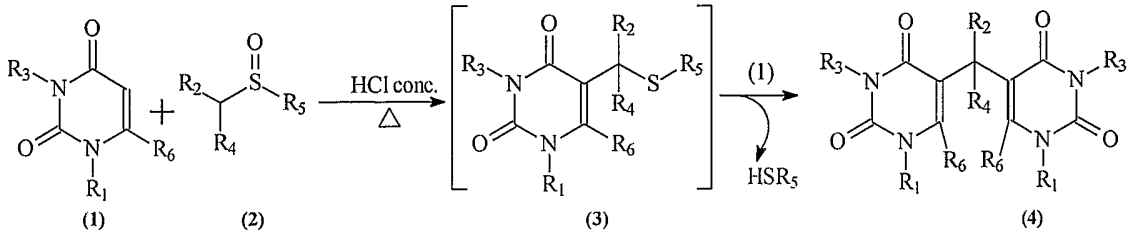
Based on experimental observations, and without wishing to be bound by theory, the following mechanisms (illustrated below) may explain the results, particularly under acidic conditions, and is potentially applicable to the reaction of any uracil analogue (unsubstituted on C-5) and any sulfoxide with at least one α-proton.



Alternatively, the mechanism may be represented by the following:



Conditions that allow or favor the resonance structure **A,** lead to the formation of product **(III)** *via* the intermediate **(II)**. This may explain why no reaction occurs under basic conditions. The intermediate **(V)**, which leads to the final product **(VI),** could be obtained by direct nucleophilic attack of **(I)** on either **(IV)**, as shown in the proposed mechanism, or **(III).** In the latter case, the mercaptan HSR_5 elimination would proceed *via* the classical SN_2 reaction mechanism. Thus, acid-catalyzed reactions of C-5 unsubstituted uracil analogs and sulfoxides bearing at least one α proton may be represented by the equation the following scheme. This reaction was used to prepare several uracil analogues (Table 2).



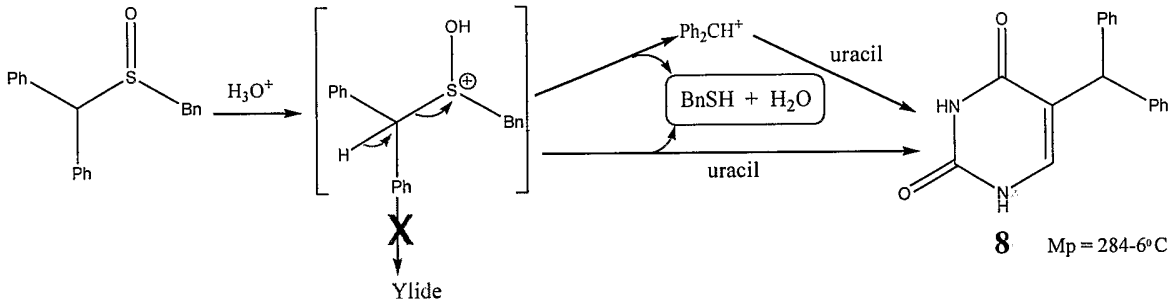
For example, concentrated hydrochloric acid (25.0 mL) was added to a flask containing 1.0 equivalent of uracil analogue **(1)** and the resulting slurry was deoxygenated by bubbling argon and vigorous stirring. The mixture was then heated in an oil bath at 75°C until a homogenous solution was obtained; then 1.5 equivalent of the sulfoxide **(2)** was added and the reaction allowed to proceed until either reagent is consumed, as by TLC. In the case of benzyl sulfoxide, an oil supernatant forms on the surface but slowly disappears as the reaction mixture gets cloudy and the product **(4)** starts to precipitate out solution. Once, the uracil analogue or sulfoxide reagent is consumed as observed by TLC, the solvent is removed in *vacuo* and the resulting slurry is treated accordingly to isolate pure products **(3)** and **(4)**. In some instances where column chromatography on silica gel was used in the purification processes, sulfoxides of product **(3)** were also isolated as minor products. Without wishing to be bound by theory, the sulfide oxidation may occur on the column since the sulfoxide is not observed on TLC in the course of reaction.

Table 2

Products from acid catalyzed reactions of uracils and sulfoxides

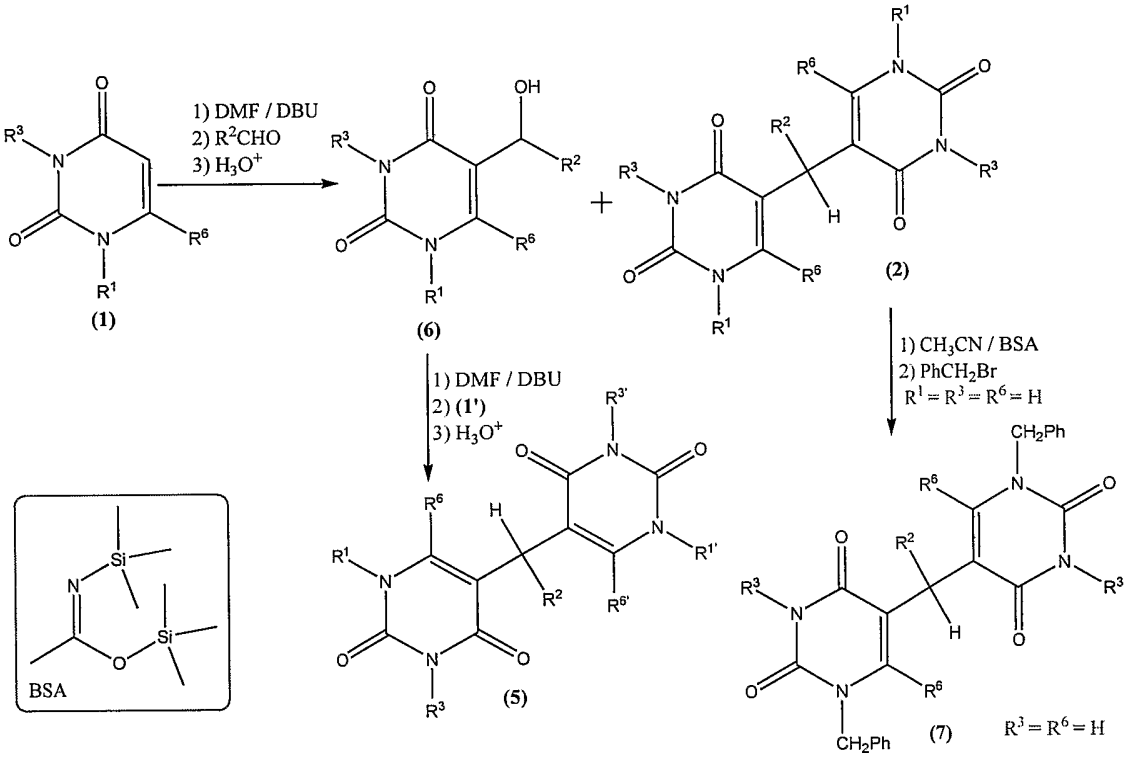
	R_1	R_2	R_3	R_4	R_5	R_6	Sulfide (3)	Sulfoxide (3′)	Product (4)
a	H	H	H	H	H	H	√	√	√
b	H	H	H	H	H	CH_3	X	X	√
c	Benzyl	H	H	H	H	H	X	X	√
d	Benzyl	H	Benzyl	H	H	H	X	X	√
e	H	H	H	Phenyl	Benzyl	H	√	√	√
f	H	H	H	Phenyl	Benzyl	CH_3	X	X	√
g	Benzyl	H	H	Phenyl	Benzyl	H	X	X	√

Though, the acid-catalyzed reactions of uracils and sulfoxides were successful with 6-methyluracil and benzylsulfoxide, it was not successful when sulfoxides bearing b-protons (Table 1A, entry g), and those able to form stable carbocations (Table 1A, entry h), which failed to yield expected products. While the reaction of butylsulfoxide yielded no uracil derivative product, that of diphenylmethylbenzylsulfoxide led to the analog **8** (below), which may form *via* the mechanism illustrated in the following scheme, bypassing the ylide formation (Pummerer reaction).



II DBU-catalyzed reactions of aldehydes and uracils

The general reaction scheme for DBU-catalyzed reactions of aldehydes and uracils may be characterized by the following reaction scheme:

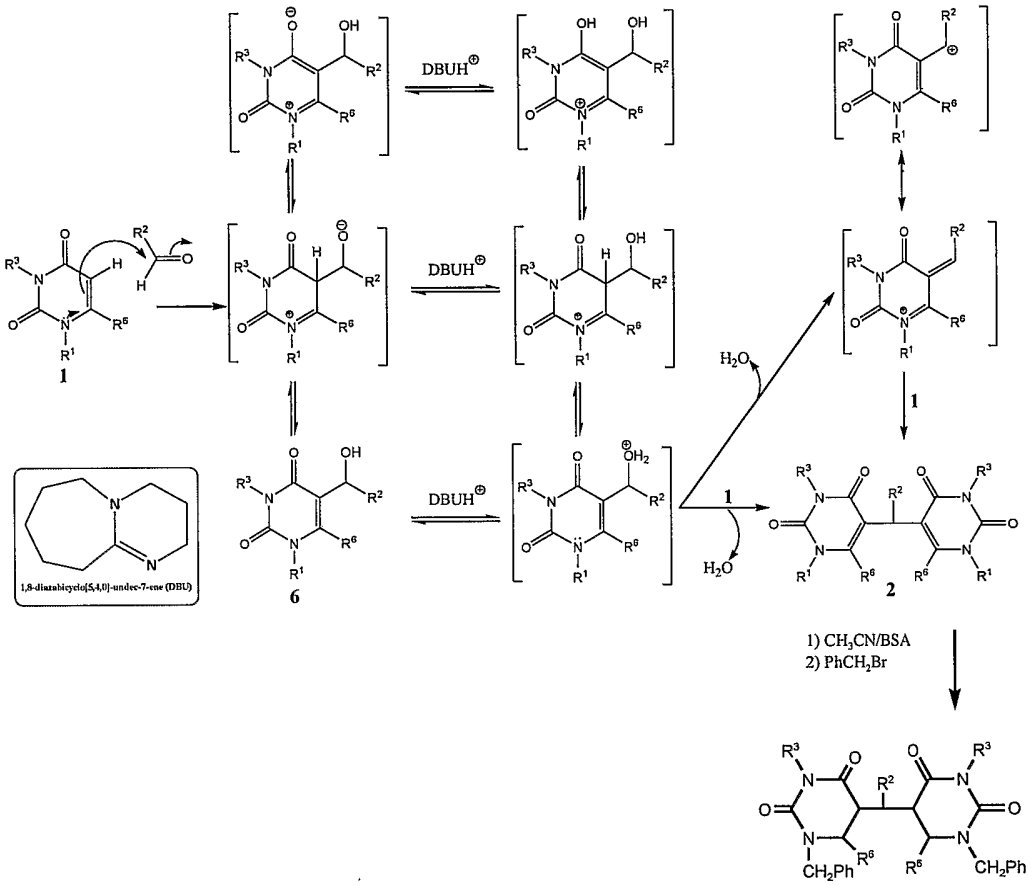


Several examples of compounds prepared under this reaction scheme are shown in Table 2A below.

Table 2A: Products from DBU-catalyzed reactions of uracils & aldehydes

	Derivative (1)	Aldehyde	Derivative (2) % Yield / Mp (°C)	Derivative (7) % Yield / Mp (°C)
d)	Uracil	Benzaldehyde	85.1 / 318-23 dec.	91.2 / 261-2
i)	Uracil	p-Chlorobenzaldehyde	82.7 / >300 dec.	79.4 / 245-7
j)	Uracil	o-Methoxybenzaldehyde	86.2 / >300 dec.	86.9 / 270-2
k)	Uracil	m-Methoxybenzaldehyde	85.0 / >300 dec.	88.4 / 260-1
l)	Uracil	p-Methoxybenzaldehyde	78.9 / >300 dec.	91.6 / 235-8
m)	Uracil	Valeraldehyde	48.7 / 360-2 dec.	84.5 / 190-2
n)	Uracil	2-Thiophene-carboxaldehyde	93.4 / 358-60	79.6 / 280-1
o)	Uracil	2-Furan-carboxaldehyde	62.9 / 287-90 dec.	92.7 / 294-6
p)	Uracil	2-Pyrrole-carboxaldehyde	0 (tar) / NA	NA
q)	Uracil	3-Indole-carboxaldehyde	0 (tar) / NA	NA
r)	Uracil	3-(1-Benzyl-2-methyl)-indole-carboxaldehyde	0 / NA	NA
s)	Uracil	3-(1-Methyl)-indole-carboxaldehyde	80.3 / 282-4 dec.	92.0 / 346-8 dec.
t)	Uracil	Phenacetaldehyde	0 / NA	NA
u)	Uracil	3-Pyridine-carboxaldehyde	96.2 / 376-8 dec.	-
v)	Uracil	1-Naphtaldehyde	85.5 / 265-70 dec.	83.7 / 246-8
w)	6-Ethyluracil	Benzaldehyde	61.3 / 329-31	-
x)	3-Benzyluracil	Benzaldehyde	87.4 / 308-10 dec.	-
y)	Uracil	p-Nitrobenzaldehyde	-	88 / -

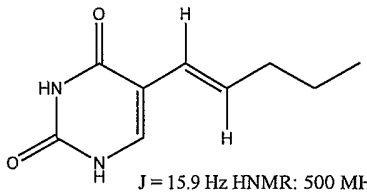
Based on experimental observations, and without wishing to be bound by theory, the following mechanisms (illustrated below) may explain the results.



The DBU-catalyzed reactions (above) of uracil derivatives and aldehydes appear to be more efficient and general with applications extending to non-aromatic aldehydes, which decompose to tarry substances under acid catalysis. Even though, these reactions are limited by steric factors as well as the thermal stability of aldehydes. Thus, unprotected *N*-pyrrole or -indole aldehydes decomposed to tarry substances under reaction conditions failing to yield the expected uracil derivatives **2** (Table 2A). Unlike phenacetaldehyde that did not decompose to tarry substances but quickly disappeared from the reaction mixture as observed by TLC, the 1-benzyl-3-carboxaldehyde-2-methylindole was quantitatively retrieved after a 24-hour reaction attempt. While the latter reaction failure may, without wishing to be bound by theory, be attributed to steric hindrance of the aldehyde substrate, the former is not yet understood although one may suspect an efficient aldol condensation that could consume the aldehyde before it could react with the nucleophilic uracil.

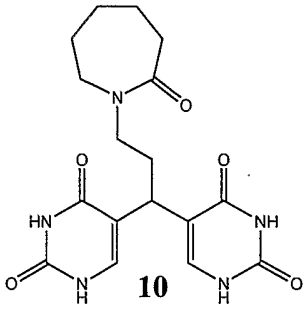
Furthermore, the reaction of valeraldehyde was also found to be slower and requiring more equivalents of aldehyde substrate to yield the expected uracil derivative **2m** (Table 2A) in addition to the alkene product **9** (below) that may have resulted from the dehydration of the alcohol **6m** (Table 2A). Attempts to extend this reaction to acetophenone (bp: 201-3°C), as an electrophile, failed to yield a uracil derivative product at 150°C in DMF, but led to an unexpected product **10** (below) when this ketone was used as solvent under reflux. Presumably, **10** derived from the addition of DBU followed by the conversion of the imine to carbonyl by hydrolysis.

Using N,O-*bis*-(trimethylsilyl)-acetamide (BSA) in acetonitrile under reflux, the 5,5'-methylene-*bis*-uracil derivatives **2** and **10** were selectively benzylated at N1 to produce the derivatives **7** and **11**, respectively, in excellent yields.



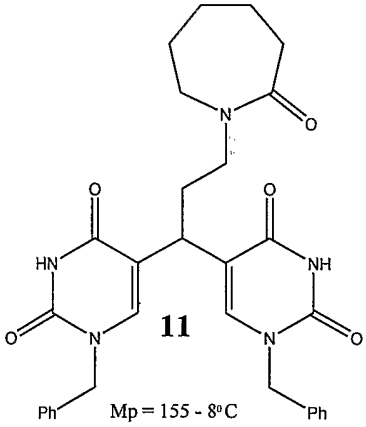
J = 15.9 Hz HNMR: 500 MHz
Mp = 272-4°C dec.

9



10

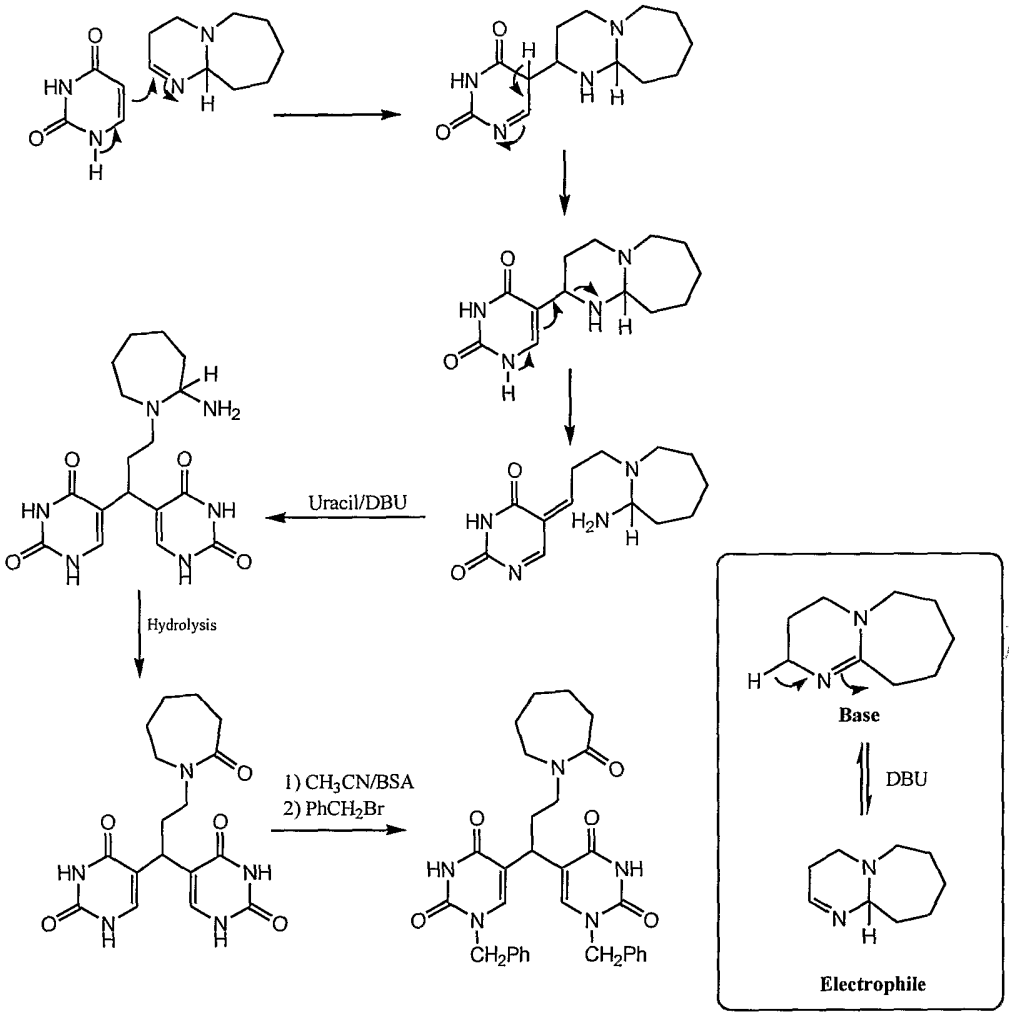
Mp > 300°C dec.



11

Mp = 155 - 8°C

Based on experimental observations, and without wishing to be bound by theory, the following mechanisms (illustrated below) may explain these results.

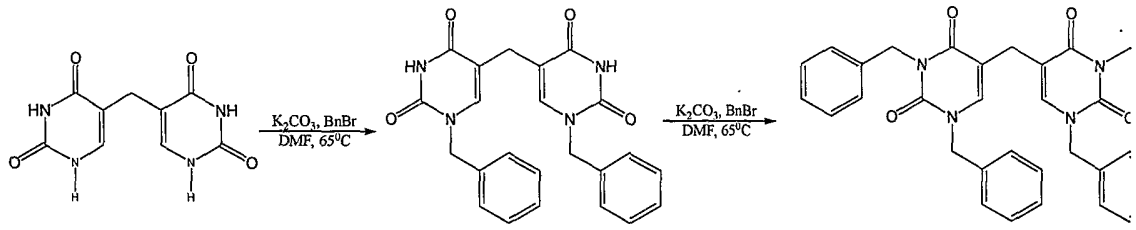


5

A. Reaction of dimethyl sulfoxide and uracil.

Following the acid-catalyzed procedure described herein, the reaction between dimethyl sulfoxide (2.0 mL, 28.1 mmol) and uracil (1.20 g, 17.8 mmol) in 20.0 mL of
10 concentrated hydrochloric acid under gentle reflux for 24 hours led to the sole product **4a** (Table 2) that was isolated as a white powder (794.0 mg, 63.8% yield). Mp: >300C (decomposes). Due to solubility problems and low volatility of compound **4a**, it was not possible to obtain accurate spectra of its samples.

4a was converted to its N-benzylated derivatives (**4c** and **4d,** Table 2A) according to the following procedure.



4a **4c** **4d**

To a mixture of compound **4a** (1.7 g, 7.2 mmol) and potassium carbonate (2.0 g, 14.4 mmol) was added 100 mL of dry dimethylformamide, and the resulting slurry was stirred at room temperature for 10 minutes under a nitrogen atmosphere. Benzyl bromide (1.7 mL, 14.4 mmol) was added and the reaction mixture was heated in an oil bath (65°C) for 22 hours. The solvent was removed under reduced pressure and ethyl acetate (100 mL) was added to the resulting slurry. After two water extractions (75 mL each), the organic phase was dried over sodium sulfate and purification by column chromatography was performed using 50% of ethyl acetate in hexanes to elute the tetra-substituted product (4d), then pure ethyl acetate to elute the di-substituted derivative (**4c**). The product **4c** was obtained as a white powder (388.9 mg, 13.0%). Mp: 274 – 278°C (decomposes). ^{1}H NMR: (300 MHz, DMSO d_6): δ 11.37 (s, 2H), 7.61 (s, 2H), 7.36 – 7.26 (m, 10H), 4.86 (s, 4H), 3.11 (s, 2H). ^{13}C NMR: δ 164.52, 151.72, 143.59, 137.83, 129.42, 128.39, 128.16, 111.00, 51.16, 24.19. MS (EI): 416.2 (11.3%), 325.2 (9.2%), 282.1 (2.6%) 91.0 (100%), 65.0 (22.7%).

The product **4d** was obtained as a white powder (1.4 g, 32.6%). Mp: 111 – 112°C. ^{1}H NMR: (300 MHz, DMSO d_6): δ 7.77 (s, 2H), 7.32 – 7.20 (m, 20H), 4.99 (s, 4H), 4.94 (s, 4H), 3.26 (s, 2H). ^{13}C NMR: δ 163.32, 151.79, 142.71, 137.98, 137.54, 129.43, 129.14, 128.43, 128.18, 127.99, 110.01, 52.50, 44.68, 25.62. MS (EI): 596.3 (14.3%), 505.3 (13.7%), 462.3 (4.8%), 91.1 (100%), 64.9 (15.6%).

B. Isolation of the sulfide and sulfoxide compounds of Table 2 in the reaction of dimethyl sulfoxide and uracil.

In order to isolate the intermediate compound **3** (Table 2), the following reaction may be performed. Under neutral conditions, a solution of uracil (4.1 g, 36.6 mmol) in DMSO (20.0 mL) was gently refluxed for 20 hours resulting into a white slurry. The reaction mixture was cooled to room temperature, and the solid filtered out, washed with acetone and dried overnight under vacuum to yield the compound **4a** (Table 2) (2.5 g, 57.2%) as a white powder. The filtrate was concentrated by distillation under reduced pressure and the resulting slurry was added to boiling ethanol containing 5.0 g of silica gel. The mixture was vigorously stirred for 15 minutes, and then prepared for chromatographic separation. After column chromatography using solvent systems of increasing polarity (ethyl acetate-hexanes 1:4; acetone; methanol) the sulfide **3a** (Table 2) and sulfoxide **3'a** (Table 2) were isolated. The compound **3a** was obtained as a white powder (639.4 mg, 10.1%). Mp: 236 - 239°C (decomposes). ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.13 (s, 1H), 10.79 (broad s, 1H), 7.38 – 7.37 (d, J = 4.25 Hz, 1H), 3.27 (s, 2H), 1.98 (s, 3H). ^{13}C NMR: δ 163.69, 151.28, 138.96, 108.96, 28.75, 14.58. High resolution MS: experimental value: 172.0300 ± 0.0008 amu, calculated: 172.0306 amu. The sulfoxide **3'a** was obtained as a white powder (128.0 mg, 1.9%). Mp: 228 - 230°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.28 (s, 1H), 11.02 (s, 1H), 7.43 (s, 1H), 3.74 - 3.68 (d, J = 12.82 Hz, 1H), 3.57 - 3.52 (d, J = 12.82 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR: δ 163.96, 151.13, 142.32, 101.86, 49.93, 37.57. MS (EI): 188.7, 171.7, 156.7, 138.7, 127.0, 125.8, 124.8, 113.7, 111.7, 92.4, 81.8, 68.1, 62.7, 55.0, 53.7.

C. Reaction of dimethyl sulfoxide and 6-methyluracil.

A solution of 6-methyluracil (5.0 g, 39.6 mmol) in dry DMSO (22.0 mL) was gently refluxed for 23 hours under a nitrogen atmosphere yielding a milky slurry that was then cooled to room temperature and poured in 20.0 mL of dichloromethane. The resulting mixture was vigorously stirred for 15 minutes and the solid was filtered, washed, and dried under vacuum overnight to give the product **4b** (Table 2) as a white powder (4.7g, 89.7%). Mp: >300C (decomposes). ^{1}H NMR: (250 MHz, DMSO d_6): δ

10.87 (s, 2H), 10.61 (s, 2H), 3.14 (s, 2H), 2.15 (s, 6H); ^{1}H NMR: (250 MHz, NaOD/D_2O): δ 3.06 (s, 2H), 1.39 (s, 6H). ^{13}C NMR (62.5 MHz, NaOD/D_2O): 178.12, 162.51, 158.94, 111.81, 22.71, 21.75. Due to low solubility in most organic solvents and low volatility of this compound, it was not possible to obtain an accurate mass spectrum.

5

D. Reaction of dibenzyl sulfoxide and uracil.

Following the acid-catalyzed procedure, a slurry mixture of uracil (2.31g, 20.6 mmol), benzyl sulfoxide (5.76g, 25.0 mmol) and concentrated hydrochloric acid (25.0 mL) was deoxygenated by bubbling argon, and then heated in an oil bath (75°C) under
10 an argon atmosphere. After 15 minutes, all the solids were dissolved yielding a brown liquid mixture with reddish supernatant oil, which progressively disappears as the reaction mixture gets cloudier and a whitish solid precipitates out solution. Total reaction time is 12 hours. The reaction slurry was added to 50.0 mL of hot acetone and the resulting mixture was vigorously stirred (15 minutes), then filtered while hot. The
15 solid product was washed with water (25 mL X 2), acetone (25 mL X 2), and ether, then dried overnight under vacuum to afford the compound **4e** (Table 2) as a white powder (1.93 g, 60.0%). Mp: 318 – 323°C (decomposes). ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.12 (s, 2H), 10.71 – 10.69 (d, J = 5.80 Hz, 2H), 7.44 – 7.14 (m, 5H), 6.78 – 6.75 (d, J = 5.80 Hz, 2H), 5.02 (s, 1H). ^{13}C NMR: (100 MHz, DMSO d_6): δ 163.50, 151.13,
20 140.38, 139.41, 128.41, 126.54, 113.18, 38.88. MS (EI): 311.9, 239.7, 212.8, 201.9, 199.9, 198.8, 170.7, 155.4, 130.0, 128.5, 111.8, 101.7, 90.7, 75.9, 68.5, 62.2.

Using a rotary evaporator, solvents were removed from the filtrate and the resulting residue mixed with acetone was adsorbed on silica gel for column chromatography eluted first with ethyl acetate-chloroform (1:1) for the sulfoxide **3'e**
25 (Table 2); then ethyl acetate-acetone (1:1) for residual uracil (755.7 mg). The product **3'e** (Table 2) was obtained as white powder (106.0 mg, 1.5%). Mp: 253 – 255°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.43 (s, 1H), 11.15 (broad s, 1H), 7.87 – 7.85 (d, J = 4.88 Hz, 1H), 7.46 – 7.32 (m, 10H), 5.62 (s, 1H), 4.65 – 4.60 (d, J = 13.42 Hz, 1H), 4.45 – 4.39 (d, J = 13.42 Hz, 1H). ^{13}C NMR: δ 163.30, 150.51, 141.31, 131.89, 131.16,
30 130.07, 128.72, 128.41, 128.05, 105.37, 61.07, 56.93.

E. Reaction of dibenzyl sulfoxide and 6-methyluracil.

The compound **4f** (Table 2) was prepared according to the acid-catalyzed procedure from benzyl sulfoxide (4.69 g, 20.4 mmol), 6-methyluracil (2.62 g, 21.6 mmol), and concentrated hydrochloric acid (50.0 mL) at 90°C. After 12 hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was refluxed in methanol (50 mL, 10 minutes), filtered to afford a white solid that was washed with chloroform, then ether, and dried under vacuum overnight to yield the product **4f** (Table 2) as a white powder (1.81 g, 51.2%). Mp: 352 – 354°C (decomposes). ^{1}H NMR: (250 MHz, DMSO d_6): δ 10.99 (s, 2H), (10.66, 2H), 7.32 (m, 5H), 5.38 (s, 1H), 1.81 (s, 6H). ^{13}C NMR: δ 164.27, 151.11, 150.78, 141.45, 128.25, 127.60, 125.88, 108.70, 32.27, 17.58. MS (EI): 341.0, 339.9, 267.8, 253.9, 214.9, 213.9, 212.8, 169.5, 142.9, 141.7, 125.7, 101.5, 90.6, 82.7, 67.6, 62.4.

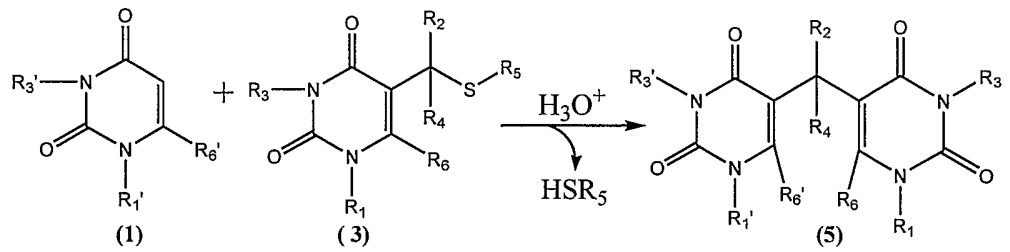
F. Reaction of dibenzyl sulfoxide and 1-benzyluracil.

Following the acid-catalyzed procedure, the compound **4g** (Table 2) was prepared from benzyl sulfoxide (3.40 g, 14.6 mmol), 1-benzyluracil (1.18 g, 5.8 mmol), and concentrated hydrochloric acid (25.0 mL) at 75°C. After 16 hours of reaction, the solvent was completely removed using a rotary evaporator and the resulting yellow solid was recrystallized from methanol, washed with cold methanol, then ether, and dried under vacuum overnight to yield the compound **4g** (Table 2) as a white powder (684.2 mg, 48.9%) melting at 256 - 258°C. ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.42 (s, 2H), 7.36 (m, 17H), 5.09 (s, 1H), 4.95 – 4.89 (d, J = 15.25 Hz, 2H), 4.82 – 4.76 (d, J = 15.25 Hz, 2H). ^{13}C NMR: δ 162.98, 150.72, 143.20, 139.82, 136.84, 128.61, 128.41, 128.33, 127.54, 127.08, 126.67, 114.32, 50.59, 39.05. MS (EI): 492.1, 405.1, 327.9, 313.9, 224.8, 200.7, 180.6, 153.7, 126.2, 114.7, 90.8, 76.6, 64.8.

G. Reaction of 5-methylthiomethyluracil and 1-benzyluracil

The proposed mechanism illustrated above suggests that all reactions proceed *via* the sulfide intermediate (**III**) in order to yield the final product 5,5'-methylene-bis-uracil analogues. This proposed mechanism was confirmed by directly reacting the sulfide

intermediate with C-5 unsubstituted uracil analogues affording unsymmetrical 5,5'-methylene-bis-uracil analogues.



	R_1	$R_{1'}$	R_2	R_3	$R_{3'}$	R_4	R_5	R_6	$R_{6'}$
a	H	Benzyl	H	H	H	H	CH_3	H	H
b	H	H	H	H	H	H	CH_3	H	Benzyl

For example, following the acid-catalyzed reaction procedure, the compound **(5a)** (above) was prepared by gently refluxing the mixture of 5-methylthiomethyluracil (622.3 mg, 3.62 mmol), 1-benzyluracil (734.5 mg, 3.64 mmol) and concentrated hydrochloric acid (17.0 mL). After 12 hours, the solvent was completely removed on a rotary evaporator, and the resulting residue was stirred in hot DMSO (90°C), and then filtered to remove the insoluble solids found to be a mixture of **(5a)** and its debenzylated product. Acetone was added to the hot DMSO filtrate until cloudy, and then allowed to slowly cool down. The resulting crystals were washed with acetone then ether, and dried overnight under vacuum to afford the compound **(5a)** as white powder (218.2 mg, 18.4%). Mp: 304 - 309°C (decomposed). ^{1}H NMR: (250 MHz, DMSO d_6): δ 11.36 (s, 1H), 11.06 (s, 1H), 10.68 (s, 1H), 7.57 (s, 1H), 7.38 – 7.15 (m, 6H), 4.86 (s, 2H), 3.078 (s, 2H). ^{13}C NMR: δ 164.24, 163.74, 151.38, 150.84, 142.52, 139.23, 137.05, 128.60, 127.55, 127.32, 110.57, 108.84, 50.33, 23.15. High resolution MS: experimental value: 326.0957±0.0008, calculated: 326.1015 amu.

H. Reaction of 5-methylthiomethyluracil and 6-benzyluracil

Following the acid-catalyzed reaction procedure, the compound **(5b)** was prepared by gently refluxing the mixture of 5-methylthiomethyluracil (308.0 mg, 1.79 mmol), 6-benzyluracil (428.8 mg, 2.12mmol) and concentrated hydrochloric acid (17.0 mL). After six hours, the solvent was completely removed on a rotary evaporator, and the resulting residue was crystallized from DMSO-Methanol. Crystals were filtered,

washed with methanol, acetone then ether, and dried overnight under vacuum to yield the compound **(5b)** as white powder. Analysis by NMR revealed that this product contained traces of the 5,5'-biuracil and required further purification.

J. Preparation of 1-benzyl-5,5'-methylene-bis-uracil (30a)

Compound 30a was prepared by gently refluxing the mixture of 5-methylthiomethyluracil (622.3 mg, 3.6 mmol), 1-benzyluracil (734.5 mg, 3.6 mmol) and hydrochloric acid (12 M, 17 mL). After twelve hours, the solvent was completely removed on a rotary evaporator, and the resulting residue was stirred in hot DMSO (10 mL, 90°C), and then filtered. Acetone was added to the hot DMSO filtrate until cloudy, and then allowed to slowly cool to room temperature. The resulting crystals were filtered, washed with acetone then ether, and dried overnight under vacuum to afford the compound 30a as white powder (218.2 mg, 18%). Mp: 304-309°C (decomposed). 1H NMR (250 MHz, DMSO d6): δ 11.36 (s, 1H), 11.06 (s, 1H), 10.68 (s, 1H), 7.57 (s, 1H), 7.38-7.15 (m, 6H), 4.86 (s, 2H), 3.078 (s, 2H). 13C NMR (62.5 MHz, DMSO d6): δ 164.2, 163.7, 151.4, 150.8, 142.5, 139.2, 137.1, 128.6, 127.6, 127.3, 110.6, 108.8, 50.3, 23.2. HRMS calculated for C16H14N4O4: 326.1015, found 326.0957.

K. Preparation of 5,5'-(para-nitrophenylmethylene)-bis-uracil (2y)

To an ice water-cooled solution of 5,5'-phenylmethylene-bis-uracil (1.78 g, 5.7 mmol) in concentrated sulfuric (20 mL), was added potassium nitrate (600.0 mg, 5.9 mmol) in small portions. The resulting solution was allowed to warm up to room temperature where it was maintained for one hour, and then heated to 80°C. After eight hours, the reaction mixture was cooled to room temperature, and then poured onto ice (100 g). The resulting fine precipitate was filtered, washed with water, acetone and ether to afford the compound 2y with traces of other nitro derivatives. Recrystallization from methanol afforded the pure derivative 2y as a light yellow powder (1.96 g, 96% yield). Mp: 348-350°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.17 (d, 2H, J=2.0 Hz), 10.80 (dd, 2H, J=2.0 Hz, 6.0 Hz), 8.15 (d, 2H, J=9.0 Hz), 7.46 (d, 2H, J=9.0 Hz), 6.94 (d, 2H, 6.0 Hz), 5.14 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.5, 151.1, 149.0, 146.2, 140.2, 129.6, 123.4, 111.9, 39.1.

L. Products from DBU-catalyzed reactions of uracil derivatives and aldehydes

I. Preparation of 5,5'-phenylmethylene-bis-uracil (2e): Protocol for the DBU-catalyzed reaction of uracil derivatives and aldehydes

To a suspension of uracil (1.2 g, 11.1 mmol) in dry DMF (10 mL), DBU (1.7 mL, 11.3 mmol) was added and the resulting mixture was stirred at room temperature until a colorless solution was formed. This solution was then heated to a gentle reflux and benzaldehyde (1.1 mL, 10.9 mmol) was added. After twenty minutes, the reaction mixture became cloudy, and then a white precipitate formed. After ten more minutes, under refluxing, the reaction was complete as indicated by TLC. The solvent was evaporated using a rotary evaporator and the resulting slurry was acidified to pH 4 using hydrochloric acid (6 M), then stirred and cooled in ice bath. The resulting fine precipitate was filtered and washed several times with water, then acetone and ether to afford the pure product 2e (1.5 g, 85% yield) as a white powder after overnight drying under vacuum. 2.1.1.1.1 Preparation of 5,5'-(para-chlorophenylmethylene)-bis-uracil (2i) Compound 2i was prepared according to the DBU-catalyzed reaction procedure starting from para-chlorobenzaldehyde (2.1 g, 15.1 mmol), uracil (1.4 g, 12.7 mmol), and DBU (1.9 mL, 12.7 mmol) in dry DMF (10 mL). After eighteen hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol and ether to afford the pure product 2i as a white powder (1.8 g, 83% yield). Mp: >300°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.09 (br s, 2H), 10.75 (br s, 2H), 7.35 (d, 2H, J=8.0 Hz), 7.19 (d, 2H, J=8.5 Hz), 6.82 (s, 2H), 5.00 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.5, 151.1, 139.7, 139.6, 131.1, 130.3, 128.3, 112.7, 38.4.

II. Preparation of 5,5'-(ortho-methoxyphenylmethylene)-bis-uracil (2j)

Compound 2j was prepared according to the DBU-catalyzed reaction procedure starting from ortho-methoxybenzaldehyde (1.8 g, 13.6 mmol), uracil (1.3 g, 12.4 mmol), and DBU (1.8 mL, 12.0 mmol) in dry DMF (10 mL). After six hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol and ether to afford the pure product 2j as a white powder (1.8 g, 86% yield). Mp: >300°C

(decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.06 (s, 2H), 10.61 (d, 2H, J=4.5 Hz), 7.26-6.87 (m, 4H), 6.64 (d, 2H, J=6.0 Hz), 5.30 (s, 1H), 3.74 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 163.5, 156.4, 151.2, 138.8, 128.3, 128.1, 128.0, 120.6, 112.8, 111.1, 55.5, 32.2.

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III. Preparation of 5,5'-(meta-methoxyphenylmethylene)-bis-uracil (2k)

Compound 2k was prepared according to the DBU-catalyzed reaction procedure starting from meta-methoxybenzaldehyde (1.7 g, 12.8 mmol), uracil (1.4 g, 12.7 mmol), and DBU (1.9 mL, 12.7 mmol) in dry DMF (10 mL). After three hours of reaction, the
10 solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol to afford the pure product 2k as a white powder (1.8 g, 85% yield). Mp: >300°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.09 (s, 2H), 10.67 (s, 2H), 7.25-7.21 (m, 1H), 6.82-6.69 (m, 5H), 4.99 (s, 1H), 3.72 (s, 3H). 13C NMR (125 MHz,
15 DMSO d6): δ 163.5, 159.3, 151.2, 142.1, 139.4, 129.4, 120.6, 114.5, 113.1, 111.7, 55.0, 38.8.

IV. Preparation of 5,5'-(para-methoxyphenylmethylene)-bis-uracil (2l)

Compound 2l was prepared according to the DBU-catalyzed reaction procedure
20 starting from para-methoxybenzaldehyde (1.6 g, 11.5 mmol), uracil (1.3 g, 11.4 mmol), and DBU (1.7 mL, 11.0 mmol) in dry DMF (10 mL). After six hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol to afford the pure product 2l as a white powder (1.5 g, 79% yield). Mp: >300°C
25 (decomposed). 1H NMR (500 MHz, DMSO d6): δ 10.84 (br s, 4H), 7.06 (d, 2H, J=8.5 Hz), 6.87 (d, 2H, J=8.5 Hz), 6.75 (s, 2H), 4.95 (s, 1H), 3.72 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 164.0, 158.4, 151.7, 139.7, 139.6, 132.6, 129.9, 114.3, 114.0, 55.5, 38.5.

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V. Preparation of 5,5'-butylmethylene-bis-uracil (2m)

Compound 2m was prepared according to the DBU-catalyzed reaction procedure starting from valeraldehyde (1.8 g, 20.7 mmol), uracil (2.3 g, 20.5 mmol), and DBU (3.1 mL, 20.7 mmol) in dry DMF (20 mL). After twenty hours of reaction, uracil was still present in the reaction mixture; thus an extra 1 mL of valeraldehyde was added and reaction continued for two more hours to complete the reaction of uracil. The solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol to afford the pure product 2m as a white powder (1.5 g, 49% yield). Mp: 360-362°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 10.96 (br s, 2H), 10.69 (br s, 2H), 7.16 (s, 2H), 3.55 (t, 1H, J=7.5 Hz), 1.69 (q, 2H, J=7.5 Hz), 1.25 (m, 2H), 1.14 (m, 2H), 0.84 (t, 3H, J=7.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 163.9, 151.1, 138.4, 112.9, 33.4, 30.7, 29.6, 21.9, 13.9.

The filtrate was mixed with silica gel and dried on a rotary evaporator. After purification by flash chromatography (CHCl3/CH3OH: 9/1), the alkene product 34 was obtained as a white powder (197.9 mg, 5% yield). Mp: 272-274°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.07 (br s, H), 10.90 (br s, H), 7.45 (s, 1H), 6.41 (dt, 1H, J=16.0 Hz, 7.0 Hz), 6.03 (d, 1H, J=16.0 Hz), 2.05 (m, 2H), 1.39 (m, 2H), 0.88 (t, 3H, J=7.0 Hz). 13C NMR (125 MHz, DMSO d6): δ 163.2, 150.6, 138.0, 129.5, 121.7, 109.7, 35.0, 22.1, 13.6. HRMS calculated for C9H12N2O2: 180.0899, found 180.0901.

VI. Preparation of 5,5'-(2-thiophen-yl-methylene)-bis-uracil (2n)

Compound 2n was prepared according to the DBU-catalyzed reaction procedure starting from 2-thiophene-carboxaldehyde (1.3 g, 12.0 mmol), uracil (1.3 g, 11.4 mmol), and DBU (1.8 mL, 12.0 mmol) in dry DMF (10 mL). After twelve hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained dark red crude product was dissolved in aqueous sodium hydroxide (20 mL, 1 M) and the resulting solution was treated with activated charcoal under reflux for thirty minutes. After hot filtration through celite, the obtained filtrate was cooled to room temperature, acidified (pH 6), and further cooled in ice bath. The resulting precipitate was filtered, washed with water, acetone, and ether. After

drying under vacuum, the product 2n was obtained as a white powder (1.7 g, 93% yield). Mp: 358-360°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.14 (s, 2H), 10.75 (br s, 2H), 7.39 (dd, 1H), 6.98-6.96 (m, 3H), 6.85 (d, 1H, J=3.5 Hz), 5.25 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.3, 151.1, 144.4, 139.4, 127.1, 125.9, 124.8, 113.3, 34.0.

VII. Preparation of 5,5'-(2-furan-yl-methylene)-bis-uracil (2o)

Compound 2o was prepared according to the DBU-catalyzed reaction procedure starting from 2-furan-carboxaldehyde (1.7 g, 18.1 mmol), uracil (2.0 g, 18.0 mmol), and DBU (2.6 mL, 17.4 mmol) in dry DMF (10 mL). After four hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained dark red crude product was dissolved in aqueous sodium hydroxide (20 mL, 1 M) and the resulting solution was treated with activated charcoal under reflux for thirty minutes. After hot filtration through celite, the obtained filtrate was cooled to room temperature, acidified (pH 6), and further cooled in ice bath. The resulting precipitate was filtered, washed with water, acetone, and ether. After drying under vacuum, the product 2o was obtained as a white powder (1.7 g, 63% yield). Mp: 287-290°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.14 (s, 2H), 10.75 (s, 2H), 7.57 (d, 1H, J=1.0 Hz), 6.91 (s, 2H), 6.38 (dd, 1H, J=3.0 Hz, 1.0 Hz), 6.13 (d, 1H, 3.0 Hz), 5.05 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.3, 153.4, 151.1, 142.4, 139.3, 111.0, 110.5, 107.6, 32.8.

VII. Preparation of 5,5'-(N-methyl-3-indol-yl-methylene)-bis-uracil (2s)

Compound 2s was prepared according to the DBU-catalyzed reaction procedure starting from 1-methyl-3-indole-carboxaldehydel (1.0 g, 6.5 mmol), uracil (1.4 g, 12.7 mmol), and DBU (1.9 mL, 12.7 mmol) in dry DMF (10 mL). After twenty-four hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained crude product was recrystallized from methanol to afford the pure product 2s as a white powder (1.9 g, 80% yield). Mp: 282-284°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.08 (s, 2H), 10.59 (br d, 2H, J=4.5 Hz), 7.39 (d, 1H, J=8.5 Hz), 7.28 (d, 1H, J=8.0 Hz), 7.14 (t, 1H, J=7.5 Hz), 7.01-

6.99 (br t, 2H), 6.80 (d, 2H, J=6.0 Hz), 5.22 (s, 1H), 3.72 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 163.6, 151.3, 138.6, 137.2, 128.6, 126.2, 121.3, 118.7, 118.7, 113.2, 113.1, 109.8, 32.3, 30.0.

IX. Preparation of 5,5'-(3-pyridin-yl-methylene)-bis-uracil (2u)

Compound 2u was prepared according to the DBU-catalyzed reaction procedure starting from 3-pyridine-carboxaldehyde (2.0 g, 18.7 mmol), uracil (2.1 g, 18.7 mmol), and DBU (140 µL, 0.9 mmol) in dry DMF (10 mL). After eight hours of reaction, the solvent was removed on a rotary evaporator to yield a yellow residue. Crystallization from methanol and dichloromethane yielded a pale yellow powder that was recrystallized from methanol to afford the pure product 2u as a white powder (2.8 g, 96% yield). Mp: 376-378°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.14 (d, 2H, J=1.5 Hz), 10.69 (br dd, 2H), 8.44-8.41 (m, 2H), 7.61 (d, 1H, J=8.0 Hz), 7.34 (dd, 1H, 8.0 Hz, 5.0 Hz), 6.90 (d, 2H, J=6.0 Hz), 5.05 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.5, 151.1, 149.5, 147.4, 139.9, 136.3, 136.2, 123.4, 112.1, 36.9.

X. Preparation of 5,5'-(1-naphth-yl-methylene)-bis-uracil (2v)

Compound 2v was prepared according to the DBU-catalyzed reaction procedure starting from 1-naphthaldehyde (3.1 g, 20.1 mmol), uracil (2.2 g, 20.1 mmol), and DBU (3.0 mL, 20.1 mmol) in dry DMF (20 mL). After eight hours of reaction, the solvent was removed on a rotary evaporator and the resulting slurry was treated as for the typical procedure. The obtained yellow crude product was dissolved in aqueous sodium hydroxide and extracted with chloroform. The organic phase was discarded and the aqueous layer was acidified (pH: 6) with hydrochloric acid to precipitate the product as a white solid. After filtration, the product was washed with water, acetone and ether, and dried under vacuum to afford the derivative 2v as a white powder (3.1 g, 86% yield). Mp: 265-270°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.19 (d, 2H, J=1.5 Hz), 10.68 (dd, 2H, J=1.5 Hz, 6.0 Hz), 7.95 (d, 1H, J=7.5 Hz), 7.94-7.79 (m, 2H), 7.57-7.51 (m, 2H), 7.45 (t, 1H, J=7.5 Hz), 7.23 (d, 1H, 7.5 Hz), 6.74 (d, 2H, J=4.5 Hz), 5.78 (s, 1H). 13C NMR (125 MHz, DMSO d6): δ 163.6, 151.2, 139.8, 136.7, 136.7, 130.7, 128.7, 127.4, 126.5, 125.8, 125.3, 124.9, 123.1, 112.5, 34.3.

XI. Preparation of 6,6'-diethyl 5,5'-phenylmethylene-bis-uracil (2w)

Compound 2w was prepared according to the DBU-catalyzed reaction procedure starting from benzaldehyde (0.8 g, 7.3 mmol), 6-ethyluracil (1.0 g, 7.3 mmol), and DBU (1.1 mL, 7.3 mmol) in dry DMF (10 mL). After twelve hours of reaction, the solvent was removed on a rotary evaporator and the resulting dark oil was treated as for the typical procedure. The obtained crude product was recrystallized from methanol / ether to afford the pure product 2w as a white powder (821.1 mg, 61% yield). Mp: 329-331°C. 1H NMR (500 MHz, DMSO d6): δ 10.94 (s, 2H), 10.57 (s, 2H), 7.26-7.09 (m, 5H), 5.51 (s, 1H), 2.36-2.17 (m, 4H), 0.77 (t, 3H, J=7.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 164.4, 155.9, 151.0, 142.5, 128.0, 127.6, 125.7, 108.4, 37.9, 24.1, 11.7.

XII. Preparation of 3,3'-dibenzyl-5,5'-phenylmethylene-bis-uracil (2x)

Compound 2x was prepared according to the DBU-catalyzed reaction procedure starting from benzaldehyde (210.0 mg, 1.98 mmol), 3-benzyluracil (401.3 mg, 2.0 mmol), and DBU (300 µL, 2.0 mmol) in dry DMF (10 mL). After twelve hours of reaction, the solvent was removed on a rotary evaporator and the resulting dark slurry was treated as for the typical procedure. The obtained crude product was dissolved in acetone and the resulting solution was treated with the decolorizing activated charcoal (Norit A) under reflux for thirty minutes. After hot filtration through a celite pad, the obtained colorless solution was concentrated on a rotary evaporator, cooled in an ice-bath to afford the pure product 2x (427.1 mg, 87% yield) as a white powder after filtration, washing with ether, and drying overnight under vacuum. Mp: 308-310°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.11 (s, 2H), 7.32-7.19 (m, 15H), 6.88 (s, 2H), 5.14 (s, 1H), 4.94 (s, 4H). 13C NMR (125 MHz, DMSO d6): δ 162.5, 151.0, 140.1, 138.3, 137.2, 128.5, 128.3, 127.5, 127.0, 126.7, 112.6, 43.0, 40.2.

XIII. Preparation of the uracil derivative (10) To a suspension of uracil (2.0 g, 17.8 mmol) in acetophenone (20 mL), DBU (2.6 mL, 17.4 mmol) was added and the resulting mixture was heated to reflux, resulting in the formation of a pink solution. After twenty minutes, the reaction mixture became cloudy, and then a white precipitate formed. Refluxing was continued (8 hours) until no uracil could be detected by TLC.

After being cooled to room temperature, the reaction mixture was poured into ether (200 mL). The resulting precipitate was filtered and washed with ether to remove the allergenic acetophenone affording a white powder. The obtained powder was mixed with hydrochloric acid (25 mL, 1 M) and stirred at 60°C for one hour. After cooling to room temperature, the fine precipitate was filtered, washed with water, acetone, and ether to afford the pure product 10 (2.6 g, 81% yield) as a white powder after overnight drying under vacuum. Mp >300 (decomposed). 1H NMR (500 MHz, DMSO d6): δ 10.95 (d, 2H, J=1.5 Hz), 10.79 (br d, 2H, J=4.5 Hz), 7.18 (d, 2H, J=6.0 Hz), 3.50 (t, 1H, J=8.0 Hz), 3.28-3.15 (m, 4H), 2.36-2.34 (m, 2H), 1.86 (q, 2H, J=7.5 Hz), 1.61-1.50 (m, 6H). 13C NMR (125 MHz, DMSO d6): δ 174.3, 163.8, 151.0, 138.7, 112.1, 48.5, 45.8, 36.6, 31.6, 29.3, 29.2, 28.3, 23.0.

M. Products from benzylation of uracil derivatives

I. Preparation of 1,1'-dibenzyl-5,5'-phenylmethylene-bis-uracil (2g): N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives

To a suspension of 5,5'-phenylmethylene-bis-uracil (2.1 g, 6.7 mmol) in dry acetonitrile (20 mL), was added BSA (6.6 mL, 27.0 mmol) and the resulting mixture was refluxed to form a colorless solution. Benzyl bromide (1.6 mL, 13.5 mmol) was added to the reaction mixture and the refluxing was continued for one hour, when the reaction was finished, as indicated by TLC and the formation of a white precipitate. After being cooled to room temperature, the reaction mixture was quenched with methanol (5 mL) and evaporated to dryness using a rotary evaporator. The resulting crude product was washed with water (25 mL), and then recrystallized from methanol, filtered, washed with ether, and dried overnight under vacuum to afford the pure compound 2g as a white powder (3.0 g, 91% yield).

II. Preparation of 1,1'-dibenzyl- 5,5'-(para-chlorophenylmethylene)-bis-uracil (7i)

Compound 7i was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(para-chlorophenylmethylene)-bis-uracil (724.9 mg, 2.1 mmol), BSA (2.2 mL, 8.8 mmol), and

benzyl bromide (550.0 μL, 4.6 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7i as white crystals (873.9 mg, 79% yield). Mp: 245-247°C. 1H NMR (500 MHz, DMSO d6): δ 11.43 (s, 2H), 7.33-7.13 (m, 16H), 5.07 (s, 1H), 4.91 (d, 2H, J=15.5 Hz), 4.82 (d, 2H, J=15.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 162.9, 150.7, 143.4, 139.0, 136.8, 131.2, 130.1, 128.5, 128.3, 127.5, 127.1, 113.8, 50.4, 38.7. HRMS calculated for C29H23N4O4Cl: 526.1407, found 526.1408.

III. Preparation of 1,1'-dibenzyl-5,5'-(ortho-methoxyphenylmethylene)-bis-uracil (7j)

Compound 7j was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(ortho-methoxyphenylmethylene)-bis-uracil (926.8 mg, 2.7 mmol), BSA (2.8 mL, 11.4 mmol), and benzyl bromide (680.0 μL, 5.7 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the typical procedure. Recrystallization from methanol / ether afforded the pure compound 7j as a white powder (1.2 g, 87% yield). Mp: 270-272°C. 1H NMR (500 MHz, DMSO d6): δ 11.37 (s, 2H), 7.33-6.83 (m, 16H), 5.28 (s, 1H), 4.92 (d, 2H, J=15.0 Hz), 4.76 (d, 2H, J=15.0 Hz), 3.60 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 162.9, 156.4, 150.8, 142.6, 136.9, 128.6, 128.2, 128.1, 127.6, 127.4, 126.9, 120.1, 110.9, 55.2, 50.1, 32.3. HRMS calculated for C30H26N4O5: 522.1903, found 522.1903.

IV. Preparation of 1,1'-dibenzyl-5,5'-(meta-methoxyphenylmethylene)-bis-uracil (7k)

Compound 7k was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(meta-methoxyphenylmethylene)-bis-uracil (949.1 mg, 2.8 mmol), BSA (2.8 mL, 11.4 mmol), and benzyl bromide (700.0 μL, 5.9 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol / ether afforded the pure

compound 7k as a white powder (1.3 g, 88% yield). Mp: 260-261°C. 1H NMR (500 MHz, DMSO d6): δ 10.26 (s, 2H), 6.59-6.40 (m, 13H), 6.09-5.94 (m, 3H), 4.56 (s, 1H), 4.43 (d, 2H, J=14.0 Hz), 4.31 (d, 2H, J=13.5 Hz), 3.27 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 163.4, 156.7, 151.2, 143.6, 141.9, 137.3, 129.9, 129.0, 128.0, 127.5, 121.0, 114.7, 114.3, 112.8, 55.3, 50.8, 39.4. HRMS calculated for C30H26N4O5: 522.1903, found 522.1901.

V. Preparation of 1,1'-dibenzyl-5,5'-(para-methoxyphenylmethylene)-bis-uracil (7l)

Compound 7l was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(para-methoxyphenylmethylene)-bis-uracil (891.7 mg, 2.6 mmol), BSA (2.6 mL, 10.6 mmol), and benzyl bromide (670.0 µL, 5.6 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7l as a white powder (1.2 g, 92% yield). Mp: 235-238°C. 1H NMR (500 MHz, DMSO d6): δ 11.39 (s, 2H), 7.33-7.10 (m, 12H), 7.0 (d, 2H, J=8.5 Hz), 6.81 (d, 2H, J=8.5 Hz), 5.02 (s, 1H), 4.90 (d, 2H, J=15.5 Hz), 4.80 (d, 2H, J=15.5 Hz), 3.71 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 162.9, 157.9, 150.7, 142.9, 136.8, 131.4, 129.3, 128.6, 127.5, 127.0, 114.7, 113.8, 55.0, 50.3, 38.3. HRMS calculated for C30H26N4O5: 522.1903, found 522.1905.

VI. Preparation of 1,1'-dibenzyl-5,5'-butylmethylene-bis-uracil (7m)

Compound 7m was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-butylmethylene-bis-uracil (670.0 mg, 2.3 mmol), BSA (2.3 mL, 9.2 mmol), and benzyl bromide (650.0 µL, 5.5 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7m as a white powder (912.9 mg, 85% yield). Mp: 190-192°C. 1H NMR (500 MHz, DMSO d6): δ 11.29 (s, 2H), 7.57 (s, 2H), 7.35-7.25 (m, 10H), 4.94 (d, 2H, J=15.5 Hz), 4.84 (d, 2H, J=15.5 Hz),

3.61 (t, 1H, 7.5 Hz), 1.70 (q, 2H, 7.5 Hz), 1.27-1.20 (m, 4H), 0.82 (t, 3H, 7.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 163.3, 150.6, 142.3, 137.0, 128.5, 127.5, 127.2, 113.7, 50.3, 33.7, 30.7, 29.5, 21.8, 13.9. HRMS calculated for C27H28N4O4: 472.2110, found 472.2107.

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VII. Preparation of 1,1'-dibenzyl-5,5'-(2-thiophen-yl-methylene)-bis-uracil (7n)

Compound 7n was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(2-thiophen-yl-methylene)-
10 bis-uracil (925.8 mg, 2.9 mmol), BSA (3.0 mL, 12.2 mmol), and benzyl bromide (780.0 μL, 6.6 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7n as white powder (1.2 g, 80% yield). Mp: 280-281°C. 1H NMR (500 MHz, DMSO d6): δ 11.47 (s, 2H), 7.37-7.18
15 (m, 13H), 6.93 (dd, 1H, J= 3.5 Hz, J=5.0 Hz), 6.78 (d, 1H, J=3.0 Hz), 5.32 (s, 1H), 4.95 (d, 2H, J=15.5 Hz), 4.81 (d, 2H, J=15.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 162.7, 150.6, 143.8, 143.1, 136.8, 128.6, 127.5, 127.1, 127.0, 125.9, 125.0, 114.3, 50.4, 34.2. HRMS calculated for C27H22N4O4S: 498.1361, found 498.1364.

20 *VIII. Preparation of 1,1'-dibenzyl-5,5'-(2-furan-yl-methylene)-bis-uracil (7o)*

Compound 7o was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(2-furan-yl-methylene)-bis-uracil (834.8 mg, 2.7 mmol), BSA (2.8 mL, 11.4 mmol), and benzyl bromide (735.0 μL, 6.2 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and
25 the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol / ether afforded the pure compound 7o as a white powder (1.2 g, 93% yield). Mp: 294-296°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.47 (s, 2H), 7.51 (br d, 1H, J=0.5 Hz), 7.35-7.19 (m, 12H), 6.34 (dd, J=3.0 Hz, J=2.0 Hz), 6.07 (d, 1H, J=3.0 Hz), 5.11 (s, 1H), 4.95 (d, 2H, J=15.0 Hz), 4.81 (d, 2H,
30 J=15.0 Hz). 13C NMR (125 MHz, DMSO d6): δ 162.7, 152.8, 150.6, 143.0, 142.5,

136.8, 128.6, 127.5, 127.1, 111.9, 110.4, 107.8, 50.4, 33.0. HRMS calculated for C27H22N4O5: 482.1590, found 482.1582.

IX. Preparation of 1,1'-dibenzyl-5,5'-(N-methyl-3-indol-yl-methylene)-bis-uracil (7s)

Compound 7s was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(N-methyl-3-indol-yl-methylene)-bis-uracil (910.0 mg, 2.5 mmol), BSA (2.4 mL, 9.8 mmol), and benzyl bromide (670.0 μL, 5.6 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7s as a white powder (1.3 g, 92% yield). Mp: 346-348°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.39 (s, 2H), 7.37-6.92 (m, 17H), 5.28 (s, 1H), 4.87 (d, 2H, J=15.5 Hz), 4.76 (d, 2H, J=15.0 Hz), 3.69 (s, 3H). 13C NMR (125 MHz, DMSO d6): δ 162.1, 150.7, 142.6, 137.3, 136.8, 128.6, 127.3, 126.9, 126.1, 121.2, 1118.8, 114.0, 112.7, 109.7, 50.2, 32.3, 30.5. HRMS calculated for C32H27N5O4: 545.2063, found 545.2059. 2.1.1.3.9 Preparation of 1,1'-dibenzyl-5,5'-(1-naphth-yl-methylene)-bis-uracil (7v) Compound 7v was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(1-naphth-yl-methylene)-bis-uracil (1.0 g, 2.8 mmol), BSA (2.7 mL, 11.0 mmol), and benzyl bromide (740.0 μL, 6.2 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from ethanol afforded the pure compound 7v as a white powder (1.25 g, 84% yield). Mp: 246-248°C. 1H NMR (500 MHz, DMSO d6): δ 11.50 (s, 2H), 7.94-6.98 (m, 19H), 5.82 (s, 1H), 4.87 (d, 2H, J=15.0 Hz), 4.75 (d, 2H, J=15.5 Hz). 13C NMR (125 MHz, DMSO d6): δ 163.0, 150.7, 143.8, 136.7, 130.6, 128.7, 128.4, 127.6, 127.3, 126.7, 126.5, 125.7, 124.9, 123.3, 113.5, 50.3, 35.0. HRMS calculated for C33H26N4O4: 542.1954, found 542.1958.

X. Preparation of 1,1'-dibenzyl-5,5'-(para-nitrophenylmethylene)-bis-uracil (7y)

Compound 7y was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from 5,5'-(para-nitrophenylmethylene)-bis-uracil (2.0 g, 5.6 mmol), BSA (5.5 mL, 22.5 mmol), and benzyl bromide (1.5 mL, 12.6 mmol) in dry acetonitrile (25 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 7y as a yellow powder (2.6 g, 88% yield). Mp: 306-308°C (decomposed). 1H NMR (500 MHz, DMSO d6): δ 11.47 (s, 2H), 8.12 (d, 2H, J=8.5 Hz), 7.43 (d, 2H, J=9.0 Hz), 7.33-7.17 (m, 12H), 5.19 (s, 1H), 4.90 (d, 2H, J=15.0 Hz), 4.83 (d, 2H, J=15.0 Hz). 13C NMR (125 MHz, DMSO d6): δ 162.9, 150.6, 148.4, 146.2, 143.9, 136.7, 129.6, 128.5, 127.4, 127.1, 123.4, 113.0, 50.5, 39.4. HRMS calculated for C29H23N5O6: 537.1648, found 537.1651.

XI. Preparation of the uracil derivative (11)

Compound 11 was prepared according to the selective N1-benzylation protocol for 5,5'-methylene-bis-uracil derivatives starting from the uracil derivative 10 (2.0 g, 5.4 mmol), BSA (5.3 mL, 21.7 mmol), and benzyl bromide (1.5 mL, 12.6 mmol) in dry acetonitrile (20 mL). After two hours, the reaction was complete and the work-up was performed according to the selective N1-benzylation protocol. Recrystallization from methanol afforded the pure compound 11 as a white powder (2.7 g, 87% yield). Mp: 155-158°C. 1H NMR (500 MHz, DMSO d6): δ 11.29 (s, 2H), 7.64 (s, 2H), 7.34-7.26 (m, 10H), 4.92 (d, 2H, J=15.5 Hz), 4.84 (d, 2H, J=15.5 Hz), 3.62 (t, 1H, 7.5 Hz), 3.27-3.18 (br m, 4H), 2.37-2.35 (br m, 2H), 1.90 (br q, 2H, J=7.5 Hz), 1.62-1.51 (br m, 6H). 13C NMR (125 MHz, DMSO d6): δ 174.3, 163.2, 150.6, 142.5, 136.9, 128.5, 127.5, 127.2, 113.2, 50.3, 48.7, 45.9, 36.5, 31.6, 29.4, 29.2, 28.3, 22.9. HRMS calculated for C31H33N5O5: 555.2482, found 555.2486.

Example 17

Biological Testing

Epilepsy is a very complex disorder of the brain and its symptoms cover a wide spectrum of seizure types and subtypes varying from simple partial seizures to generalized seizures. In an attempt to address as many seizure variations as possible in this complex disease, testing of the compounds described herein is performed in a variety of seizure models. These models include pilocarpine induced-seizures (PIS) model, the spontaneous recurrent seizure (SRS) model, the neurotrauma or brain trauma model (BTM), maximal electroshock induced-seizures (MES), subcutaneous pentylenetetrazole induced-seizures (PTZ) model and the hippocampal kindling seizure model.

Pilocarpine induced-seizure (PIS) model

Generally, the test compound is dissolved in a mixture of saline (NaCl 0.9% by mass) and DMSO. The concentration of DMSO is normally 10% by volume. However, in cases where the test compound is very insoluble in this solvent mixture, the concentration of DMSO may be increased to a maximum of 50%. This biological test is performed following the procedure disclosed in PCT publication WO 98/40055 (Queen's University at Kingston). In this model, seizures are chemically induced by Pilocarpine and the biological test is performed and evaluated or scored as follows:

- Male Sprague-Dawley rats are injected with the test compound (100 mg/kg i.p. in saline/10% DMSO)
- The animals are observed for 15 minutes
- The animals are injected with pilocarpine (350 mg/kg i.p.)
- The animals are observed for a maximum of 45 minutes for seizure onset
- If there is any observable seizure? Was the seizure onset delayed? How severe was the seizure?
- Euthanasia

The observed seizures are scored 1 to 5 with increasing severity of symptoms.

- Score 1: ear twitch
- Score 2: score 1 plus eye squint

- Score 3: score 2 plus tail up
- Score 4: score 3 plus head jerking
- Score 5: score 4 plus falling over the side and failing to return to normal position

Spontaneous recurrent seizure (SRS) model

This model is intended to evaluate the anti-epileptogenic properties of test compounds. Moreover, the "spontaneous recurrent seizures" (SRS) model of epilepsy is further described in, *e.g.*, Mello, E. *et al.*, *Epilepsia* (1993) 34:985; Cavalheiro, J. *et al.*, *Epilepsia* (1991) 32:778. The test compound is dissolved in saline/DMSO solvent system as in the pilocarpine induced seizure model and the biological test is performed as follows:

- Male Sprague-Dawley rats are injected with Methyl-scopolamine (1 mg/kg i.p. in saline/10% DMSO)
- The animals are injected with pilocarpine (380 mg/kg i.p.), which puts the animals in status epilepticus
- 180 minutes after seizure onset, Diazepam is administered (4 mg/kg i.p.) to stop the seizures
- If the animals appear weak they are hand-fed with 20 % sucrose Gatorade solution
- Test compound is injected 1/day for 7 days (20 mg/kg i.p.)
- Three weeks later, the animals are videotaped for 8 hours/day, 5 days/week for 2 months
- The numbers of seizures are counted from the videotapes viewed by individuals blinded to the test drug/control information
- The animals are anesthetized with sodium pentobarbital, 5 mL of blood are collected from the aorta
- The animals are euthanized by cardiac puncture.
- Fixative solution is perfused to prepare the brain for histological analysis
- Brains are stored in paraffin

Neurotrauma or Brain trauma model (BTM)

BTM is a model that attempts to mimic the post-traumatic epilepsy, which is thought to result from the cascade of biochemical phenomena that follows a head (brain) injury. The solution of the test compound is prepared as in PIS or SRS models, and the biological test is performed as follows:

- Male Sprague-Dawley rats are anaesthetized with sodium pentobarbital
- The animals are placed in stereotactic head frame
- Rectal thermostat and heat lamp are set up
- Midline incision is made on scalp
- Scalp is exposed
- Skull plate & membranes are removed over target area
- 30 μL tail vein blood is removed
- 10 μL blood is injected 2 mm below brain surface over 10 minutes
- Skull plate is replaced, scalp is closed
- Animal is removed from head frame
- Test compound is injected 1/day for 7 days (20 mg/kg i.p.)
- Two weeks later, animal are videotaped for 8 hours/day, 5 days/week for 4 weeks
- The numbers of seizures are counted from the videotape viewed by individuals blinded to the test drug/control information
- The animals are anesthesized (sodium pentobarbital), 5 mL of blood collected from the aorta
- The animals are euthanized by cardiac puncture and the brains are prepared for histological analysis
- The brains are stored in paraffin waiting for histological analysis

Maximal electroshock induced-seizure (MES) model

The MES model is described in, *e.g.*, "Molecular and Cellular Targets for Anti-Epileptic Drugs" G. Avanzini, *et al.* (1997) John Libbey & Company Ltd., pp 191-198; Chapter 16, "The NIH Anticonvulsant Drug Development (ADD) Program: preclinical anticonvulsant screening project," by James P. Stables and Harvey J. Kupferberg.

In this model, seizures are induced in animals *via* electrical stimulation following the established NIH/ADD program protocol. Corneal electrodes primed with a drop of the electrolyte solution (9 % aqueous sodium chloride) are applied to the animal eyes and an electrical stimulus (50 mA in mice, 150 mA in rats; 60 Hz) is delivered for 0.2 seconds at the time of peak effect of the test compound. The animal is restrained by hand and released at the moment of stimulation to allow observation of the entire seizure. The test compound is considered to have offered protection against MES-induced seizure spread if there is abolition of the hind-leg tonic extensor component *i.e.* the hind-leg tonic extension does not exceed a 90° angle to the plane of the body.

Subcutaneous pentylenetetrazole induced-seizure (PTZ) model

In this test, the convulsant dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) is injected at the time of peak effect of the test compound. Then, the animals are isolated and observed for a period of 30 minutes to see if seizures occur. Absence of clonic spasms persisting for five seconds or longer is an indication that the test compound may have the ability to increase the pentylenetetrazole induced-seizure threshold.

Hippocampal kindling seizure model

This model is particularly useful as it, not only, provides an experimental model of seizures but also offers a means of studying the complex brain networks that may contribute to the spread and generalization of seizures from the focus, *i.e.*, epileptogenesis. Biological testing using hippocampal kindling seizure model follows the Lothman procedure (Lothman, *et al.*, Epilepsy Res., 1988, 2:366) or the Silver procedure (Silver, *et al.*, *Ann. Neurol.* (1991) 29:356). *See also*, Lothman, et al., Brain Res., 1994, 649:71, and Racine, et al., Clin. Neurophysiol., 1972, 32:281. This test is performed according to the procedure by Lothman [104] as summarized below:

- A bipolar stimulating electrode is stereotactically implanted in the ventral hippocampus (AP-3.6, ML 4.9, DV-5.0 from dura, incisor bar +5) of adult male Sprague-Dawley rats (250 – 300 g) under ketamine-xylazine anesthesia.

- Three anchor screws are attached to the skull and the electrode assembly anchored to the skull with dental acrylic cement. After the incision is closed with sutures, the animal receives a single dose of the Penicillin salt Bicillin, also known as Permapen, (60,000 units, i.m.) and is returned in its home cage to the animal quarters. Animals are kindled according to the Lothman & Williamson procedure [105].
- Briefly after one week, animals are stimulated with suprathreshold trains of 200 μA for 10 seconds, 50 Hz, every 30 minutes for six hours on alternate days until the animals are fully kindled.
- One week later, the effect of a single dose of test substance (50 mg/kg) on the behavioral seizure score (BSS) and afterdischarge duration (ADD) is assessed in a single group of kindled rats (n = 6 or 8) at time intervals of 15, 45, 75, 105, 135, 165 and 195 minutes after the test compound administration.
- Results obtained at the various time points are compared with the last control stimulus delivered 15 minutes prior to test-compound administration. Thus, each animal serves as its own control. Depending on observable symptoms, seizures are scored 1 to 5 [106].
    - Stage 1: Mouth and facial clonus
    - Stage 2: Stage 1 plus head nodding
    - Stage 3: Stage 2 plus forelimb clonus
    - Stage 4: Stage 3 plus rearing
    - Stage 5: Stage 4 plus repeated rearing and falling

If a test-compound is found to significantly lower the seizure score and decrease the afterdischarge duration, a dose-response study is initiated to quantify the drug-efficiency by varying the dose between 0 and 100% effect. Usually, the same group of kindled animals is used for multiple dose study as long as animals are permitted to dry-out for 4-5 days between studies. Test-compounds that lower the behavioral seizure score to 2 or less, and significantly decrease the afterdischarge duration are considered active or protective, and may be expected to be clinically useful against focal seizures that evolve to secondarily generalized seizures.

Six Hertz psychomotor seizure test

Although, MES and PTZ are very efficient in identifying compounds that may be useful for the treatment of the human generalized seizures, they may miss novel drugs that could be effective in the treatment of resistant partial epilepsy [107, 108]. An example of one such drug was Levetiracetam (UCB LO59), which was found to be active in the 6 Hz test but inactive in both MES and PTZ models. Seizures induced by the low frequency (6 Hertz) seem resistant to phenytoin and other sodium ion channel blockers, but sensitive to anticonvulsant drugs such as barbiturates, benzodiazepines, succinamides, and valproic acid that raise seizure threshold.

In this model, mice are pretreated intraperitoneally with 200 mg/kg of the test compound, and then challenged, after a certain interval of time (0.25, 0.5, 1.0, 4.0 h), with current (32 mA, 6 Hertz for 3 seconds) delivered through corneal electrodes to elicit a psychomotor seizure, which is characterized by a minimal clonic phase that is followed by sterotyped automatistic behaviors that resemble the aura observed for human patients with partial seizures. Subject animals that do not display these symptoms are considered protected, and the test compound active.

Neurotoxicity screen

Preliminary neurotoxicity induced by the test compound may be characterized by neurologic abnormalities and poor performances in given tasks. For instance, the neurologic deficit may be indicated by the inability to maintain equilibrium for one minute in each of three trials on a knurled rod rotating at 6 rpm. Other tests for neurotoxicity include the rotorod ataxia test, positional sense test, and gait and stance test. In the positional sense test, one hind leg is gently lowered over the edge of a table. The animal quickly reacts by returning the leg to the normal position. Failure to do so rapidly, indicates a neurologic deficit. In the gait and stance test, the neurologic deficit is indicated by a circular or zigzag gait, ataxia, and other abnormal events that may include abnormal spread of legs, body posture, tremor, hyperactivity, lack of exploratory behavior, somnolence, stupor, and catalepsy.

Biological testing results

Initial testings (PTZ, MES, and Neurotoxicity) were carried out in 12 animals at doses of 30, 100, 300 mg/kg *i.e.* 4 animals per test, at interval times of 30 minutes and 4 hours after administering the test compound. The uracil analogues, compounds 1-80, evaluated for biological activity (shown below) are presented with their structural formulae and reference numbers above; and their *in vivo* biological test results are given in Tables 3 and 4.

Note that, on average, 83.3 % of control animals develop seizures in the PIS model with a seizure onset time average of 11.6 ± 7.2 minutes, which corresponds to approximately 25.7 ± 16.0 % protective effect as calculated according to the formula in Table 3. In the interpretation of our results, compounds showing more than 50% protective effect in this test are considered bioactive and therefore protective against Pilocarpine induced-seizures. Thus, in our preliminary tests, compounds protecting at least two animals out of four in the PIS test, or delaying seizure onset more than 50% are considered biologically active and acceptable for further investigation, *e.g.*, on closely related structures, especially those analogues with minimum toxicity at therapeutic dosages.

While the limitations of these tests are not overlooked, the results from these models can be an excellent predictor of efficacy in the treatment of human seizure disorders. In fact, there exists a very strong correlation between these tests and the human forms of epilepsy. For instance, test compounds that exhibit significant biological activity in PIS, MES, PTZ or kindling models may be clinically efficacious against complex partial seizures, primary generalized seizures, absence seizures or partial seizures with secondary generalization, respectively [109-111]. However, due to technical considerations, there are significant limitations in comparing the biological test results of uracils with the literature data of conventional anticonvulsant drugs; but data given in Table 5 may offer a sense of the potential of the uracil-based compounds of the invention.

Table 3

Preliminary biological test results of uracils in the PIS model

Compound reference #	% Protective effect
1	++
2	++
3	++
4	++
5	+
6	N/A
7	N/A
8	++
9	++
10	N/A
11	++
12	++
13	-
14	++
15	-
17	+
18	++
19	+
20	+
21	++
22	+
23	+
24	-
25	++
26	+
27	++
29	+
30	+
31	++
32	++
33	+
34	++
35	-
36	+
38	++
39	++
41	+
42	N/A
43	++
44	N/A
45	N/A
46	++
47	-
48	+
49	+
50	+
52	++
53	+
54	N/A

58	-
59	+
60	+
61	+
65	+
66	-
67	+
68	++
70	-
71	++
75	N/A

Key:

-	Percentage Protective Effect < 30%
+	30% ≥ Percentage Protective Effect > 60%
++	60% ≥ Percentage Protective Effect
N/A	Not Available

Table 4

Preliminary biological test results of uracil in the PTZ-, MES-induced seizure models and neurotoxicity screens

	# animals protected in MES						# animals protected in PTZ						# animals showing Neurotoxicity					
Dose (mg/kg)	30		100		300		30		100		300		30		100		300	
Time (hrs)	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0	0.5	4.0
Cpd. # 1	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**2/8**	0/4	0/4	0/2
2	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
3	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
4	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
7	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
8	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
9	0/1	0/1	0/3	**2/3**	0/1	**1/1**	0/1	0/1	0/1	0/1	-	-	0/4	0/2	**1/8**	0/4	**3/4**	**1/2**
10	0/1	0/1	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**2/8**	**1/4**	0/4	0/2
11	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	1/5	1/1	0/4	0/2	0/8	0/4	1/4	2/2
12	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	2/8	0/4	0/4	0/2
13	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**3/8**	0/4	**3/4**	0/2
14	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
15	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/4**	0/2	0/8	0/4	**1/4**	0/2
16	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**1/8**	**1/4**	**2/4**	**1/2**
17	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**2/8**	**2/4**	0/4	0/2
19	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	**1/4**	**1/4**	0/2
20	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	**5/5**	0/1	**1/1**	**1/1**	0/4	0/2	**7/8**	0/4	**4/4**	**2/2**
21	0/4	**3/4**	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	**4/5**	0/1	0/4	0/2	0/8	0/4	0/4	0/2
22	0/1	0/1	**2/3**	0/3	**1/1**	**1/1**	0/1	0/1	**5/5**	0/1	**5/5**	1/1	0/4	0/2	**1/8**	0/4	**4/4**	0/2
23	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	**5/5**	0/1	**1/1**	0/1	0/4	0/2	**3/8**	0/4	**4/4**	0/2

	# animals protected in MES						# animals protected in PTZ						# animals showing Neurotoxicity					
24	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
25	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	1/5	0/1	0/4	0/2	0/8	0/4	0/4	0/2
26	0/1	0/1	**3/3**	0/3	**1/1**	**1/1**	0/1	0/1	0/1	0/1	**1/1**	**1/1**	0/4	0/2	**1/8**	0/4	**4/4**	0/2
27	0/1	0/1	0/3	0/3	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	**2/5**	0/4	0/2	**2/8**	0/4	**2/4**	0/2
29	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/5	0/4	0/2	0/8	0/4	0/4	0/2
30	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
31	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**1/8**	0/4	**1/4**	0/2
32	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
33	0/1	0/1	0/3	0/3	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
34	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	1/8	0/4	0/4	0/2
35	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	1/8	0/4	0/4	0/2
36	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
37	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	1/4	0/2
38	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**1/8**	0/4	**1/4**	0/2
41	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	**1/5**	0/1	0/1	**2/5**	0/4	0/2	**1/8**	0/4	**2/4**	0/2
43	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	1/4	0/2
44	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	1/8	0/4	0/4	0/2
45	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	1/5	0/1	0/1	0/1	0/4	0/2	1/8	0/4	0/4	0/2
46	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	1/8	0/4	0/4	0/2
48	0/1	0/1	**1/3**	0/3	**1/1**	**1/1**	**1/5**	0/1	**3/5**	0/1	**4/5**	**1/1**	0/4	0/2	**2/8**	0/4	**2/4**	**1/2**
49	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
50	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	**1/2**	**2/8**	**1/4**	**3/4**	0/2
52	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	**4/5**	0/1	**1/1**	0/1	0/4	0/2	**1/8**	0/4	**4/4**	0/2
54	0/1	0/1	**1/3**	0/3	**1/1**	0/1	0/1	0/1	0/1	0/1	**4/5**	0/1	0/4	0/2	0/8	0/4	0/4	0/2
56	0/1	0/1	0/3	**1/3**	0/1	**1/1**	0/1	0/1	0/1	0/1	**2/5**	0/1	0/4	0/2	0/8	0/4	0/4	**1/2**

	# animals protected in MES						# animals protected in PTZ						# animals showing Neurotoxicity					
58	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**2/5**	0/4	0/2	**3/8**	0/4	0/4	0/2
59	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
60	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	1/4	0/2
61	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2
65	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/4**	**1/2**	**2/8**	0/4	**1/4**	0/2
66	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**2/8**	**1/4**	**1/4**	0/2
67	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**4/8**	**1/4**	0/4	**1/2**
68	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	**5/8**	0/4	**2/4**	**1/2**
75	0/1	0/1	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/4	0/2	0/8	0/4	**3/4**	**1/2**

Table 5
Biological effects (median effective dosage (ED_{50}) and median toxic dosage (TD_{50})) of selected uracil analogs, conventional anticonvulsant drugs [112]

Drug name or compound ID #	MES	PTZ	Electroshock Kindling	Impairment of rotarod performance
	ED_{50} (mg/kg, i.p.) (Low - High)			TD_{50} (mg/kg, i.p.)
Levetiracetam	>540	>540	7 (2 – 10)	1060 (335 – 2081)
Valproate	188 (154 - 216)	106 (64 – 170)	66 (52 – 83)	206 (132 – 261)
Phenobarbital	12 (9 - 16)	11 (7 – 18)	12 (8 - 17)	24 (17 – 34)
Clonazepam	>3	0.02 (0.01 – 0.04)	0.03 (0.02 – 0.05)	0.8 (0.2 – 2.0)
Ethosuximide	>452	126 (78 – 196)	>254	
Carbamazepine	6 (5 - 9)	>42	6 (4 – 10)	32 (22 – 59)
Phenytoin	6 (4 - 10)	>45	6 (1 – 16)	
Vigabatrin	>2325	>2325	210 (84 – 5958)	
Lamotrigine	3 (2 - 4)	>82	4 (2 – 7)	
Gabapentin	90 (63 - 132)	>665	55 (30 – 95)	
Topiramate	12 (8 - 21)	>340	>109	
Analog (22)	14.51	25.18	69.49 (25 – 100)	>300
Analog (21)		40.4 (29.79 – 50.78)		
Analog (48)	48.9 (25.42 – 66.51)	26.79 (11.63 – 41.18)		
Analog (54)	39.46 (21.84 – 61.33)	(25 – 50)		

Note that all the data values for uracil analogs as given in Table 5 were estimated with 95% confidence interval. In both pentylene tetrazole-induced seizure and maximum electroshock-induced seizure models, the time of peak effect for the uracil analog (22) was estimated to six hours in rats, and neurotoxicity tests were extended from 0 to 24 hours.

Table 6
Biological test results of the uracil analog (22) in the pilocarpine-induced seizure model at different time intervals (T) of pilocarpine administration to rats [98].

Time (T) in hours	# Animals protected	Seizure delay time t (min.)	% Protective effect	Remarks
0.25	1/4	82	45.6	Seizure levels: 2, 4, 4
1.0	3/4	145	80.5	Seizure level: 4
4.0	2/4	121	67.2	Seizure levels: 3, 4
6.0	0/4	62	34.4	Seizure levels: 1, 4, 4, 4
Carbamazepine	4/8	248	68.9	Seizure levels: 1, 1, 2, 2 Pilocarpine administered after 0.25 hour
Phenytoin	4/8	265	73.6	Seizure levels: 4, 4, 4, 4 Pilocarpine administered after 0.25 hour
Pilocarpine	1/6	103	38.1	All 5 rats had 2 or 3 seizures of level 4 in the first 25 minutes

Biological data in Tables 5 and 6 indicate that uracil analogs compare fairly well with established anticonvulsant drugs. For example, the analog (22), shows good biological activity in various animal models including MES, PTZ, Kindling, PIS, and virtually no neurotoxicity at dosage as high as 300 mg/kg, outperforming first-line drugs of choice such as phenytoin, clonazepam, carbamazepine, and phenobarbital. Showing activity in diverse biological tests, and offering high therapeutic indexes, the uracil-based drugs are expected to offer protection over a wide range of seizure types and sub-types, with very little risk for unwanted side effects.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, *etc.*, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, *e.g.*, in ages of subject populations, dosages, and blood levels, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are within the scope of the present application.

INCORPORATION BY REFERENCE

The contents of all references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference. The appropriate components, processes, and methods of those patents, applications and other documents may be selected for the present invention and embodiments thereof.

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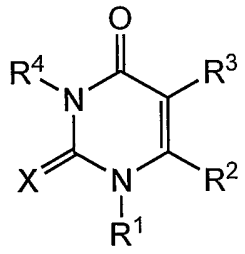
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CLAIMS

What is claimed is:

1. A compound having the formula



wherein

X is S or O;

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts or esters thereof.

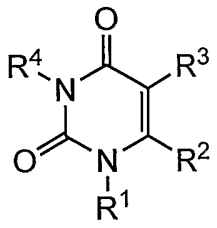
2. The compound of claim 1, wherein at least one of R^1, R^2, R^3 , or R^4 is not hydrogen.

3. The compound of claim 1, wherein at least two of R^1, R^2, R^3 , or R^4 are not hydrogen.

4. The compound of claim 1, wherein at least three of R^1, R^2, R^3 , or R^4 are not hydrogen.

5. The compound of claim 1, wherein R^1, R^2, R^3 , and R^4 are not hydrogen.

6. The compound of claim 1, wherein the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

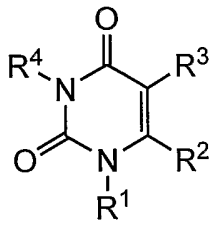
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3, -C≡CC_4H_9, -CH_2N(H)-phenyl, -CH_2N(H)CH_2-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts or esters thereof.

7. The compound of claim 6, wherein the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl;

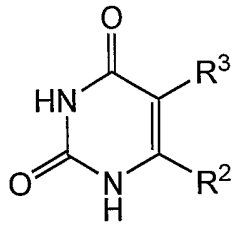
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, and n-butyl;

R^3 is selected from the group consisting of H, Br, I, and -C≡CC_4H_9; and

R^4 is selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts or esters thereof.

8. The compound of claim 6, wherein the compound is



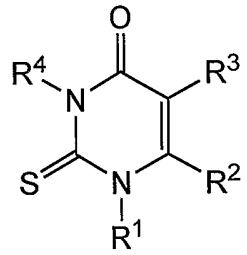
wherein

R^2 is selected from the group consisting of H, phenyl, benzyl, and -$CH_2CH(OH)$-phenyl; and

R^3 is selected from the group consisting of H, Br, -$CH_2N(H)$-phenyl, and -CH_2-O-benzyl;

or acceptable salts or esters thereof.

9. The compound of claim 1, wherein the compound is



wherein

R^1 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

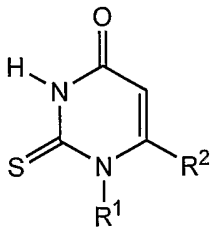
R^2 is selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -$CH_2CH(OH)$-phenyl;

R^3 is selected from the group consisting of H, Br, I, CH_3,-C≡CC_4H_9, -$CH_2N(H)$-phenyl, -$CH_2N(H)CH_2$-phenyl, -CH_2-O-benzyl, an alkylsulfide, and an alkyluracil; and

R^4 is selected from the group consisting of H, benzyl, isopropyl, p-NO_2-benzyl, and benzoyl;

or acceptable salts or esters thereof.

10. The compound of claim 9, wherein the compound is



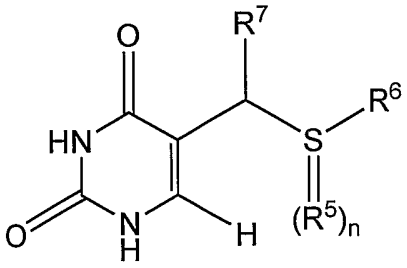
wherein

R^1 is selected from the group consisting of H and $-CH_2SCH_3$; and

R^2 is selected from the group consisting of H, n-butyl, ethyl, n-propyl, phenyl, and benzyl;

or acceptable salts or esters thereof.

11. The compound of claim 1, wherein the compound is



wherein

n is 0 or 1;

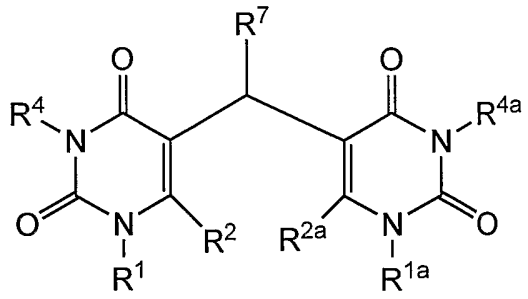
R^5 is O or absent;

R^6 is selected from the group consisting of CH_3 and benzyl; and

R^7 is selected from the group consisting of H and phenyl;

or acceptable salts or esters thereof.

12. The compound of claim 1, wherein the compound is a formula of



wherein

R^1 and R^{1a} are independently selected from the group consisting of H, benzyl, p-NO_2-benzyl, benzoyl, and -CH_2SCH_3;

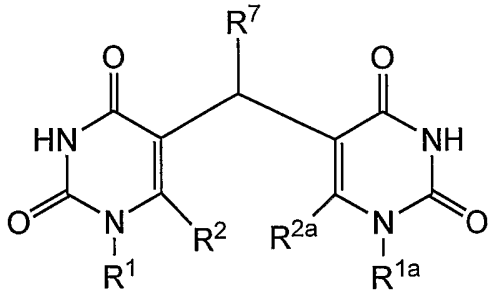
R^2 and R^{2a} are independently selected from the group consisting of H, CH_3, -CH_2CH_2-phenyl, n-butyl, ethyl, n-propyl, phenyl, benzyl, and -CH_2CH(OH)-phenyl;

R^4 and R^{4a} are independently selected from the group consisting of H, benzyl, p-NO_2-benzyl, and benzoyl; and

R^7 is selected from the group consisting of H and phenyl;

or acceptable salts or esters thereof.

13. The compound of claim 12, wherein the compound is a formula of



wherein

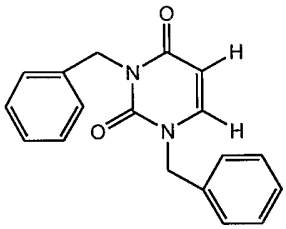
R^1 and R^{1a} are independently selected from the group consisting of H and benzyl;

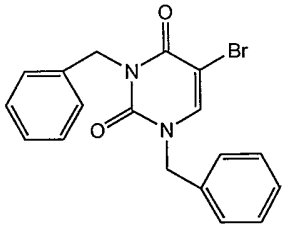
R^2 and R^{2a} are independently selected from the group consisting of H, CH_3, and benzyl; and

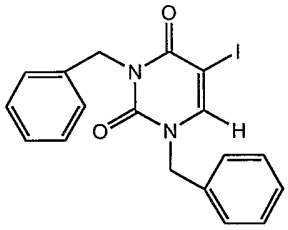
R^7 is selected from the group consisting of H and phenyl;

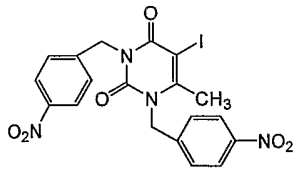
or acceptable salts or esters thereof.

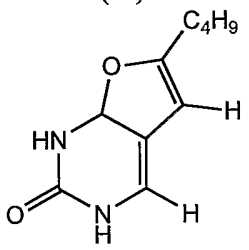
14. The compound of claim 1, wherein the compound is selected from the group consisting of

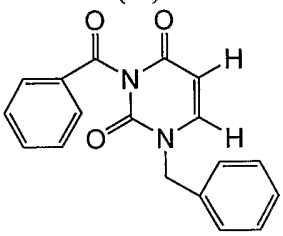

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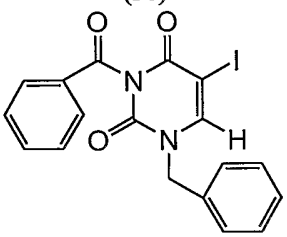

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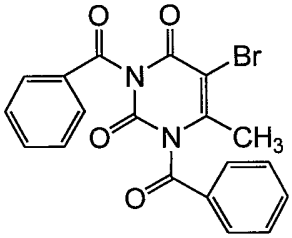

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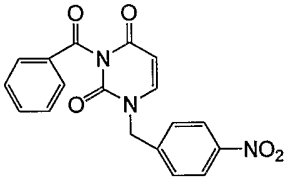

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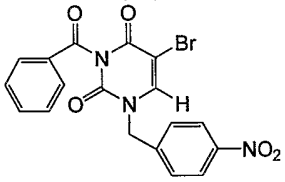

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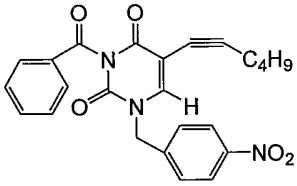

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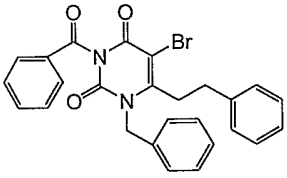

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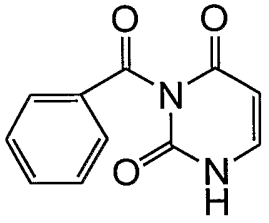

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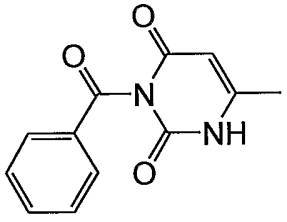

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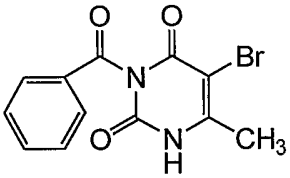

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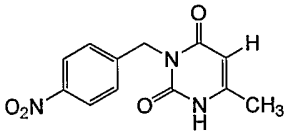

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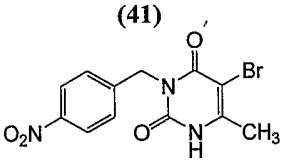

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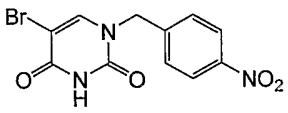

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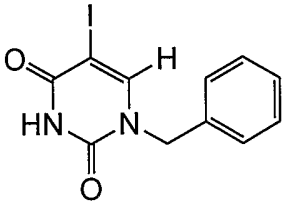

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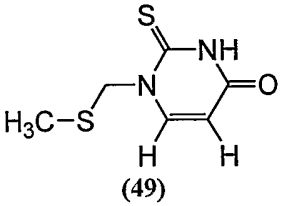

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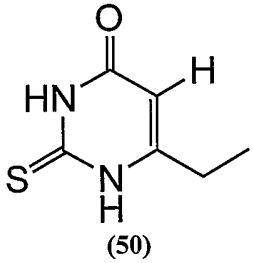

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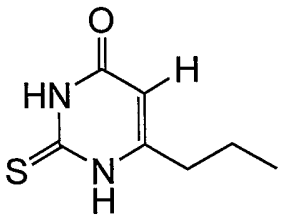

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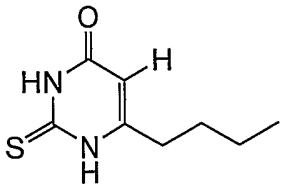

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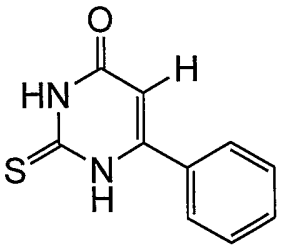

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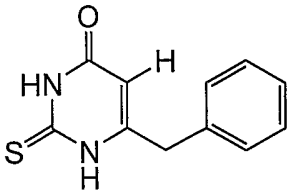
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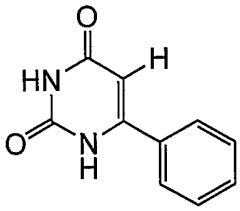
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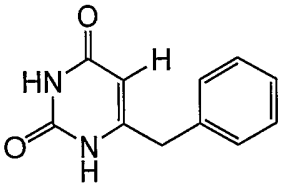
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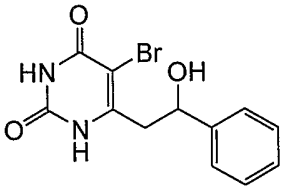
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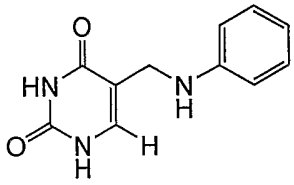
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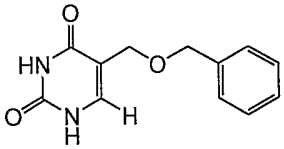
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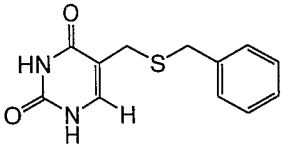
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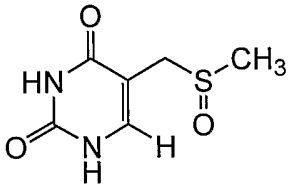
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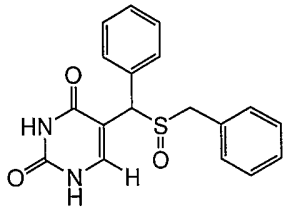
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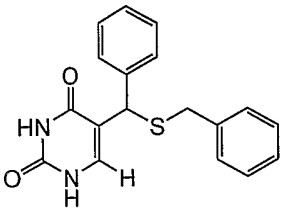
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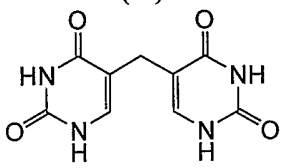
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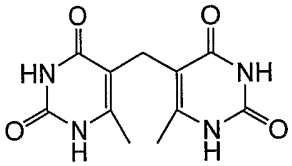
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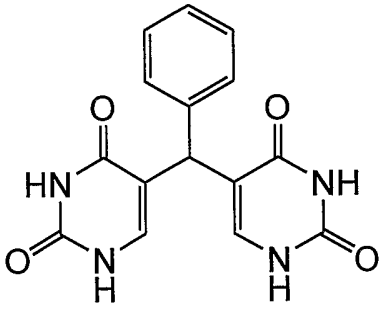
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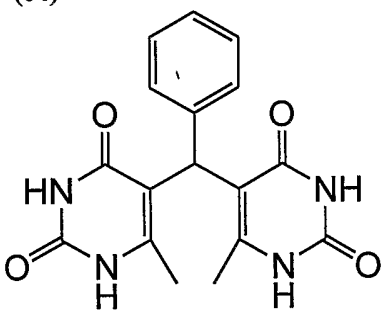
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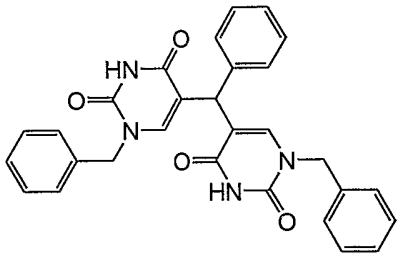
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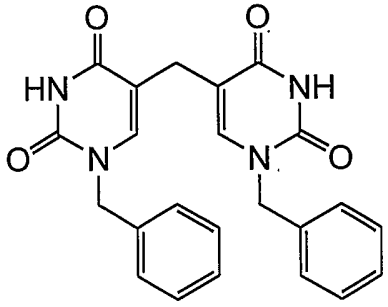
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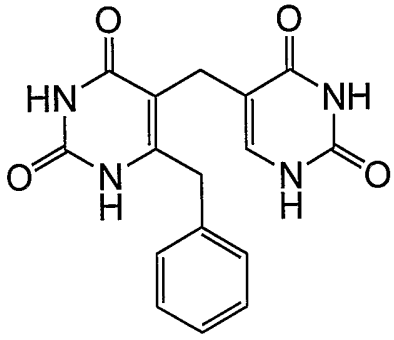
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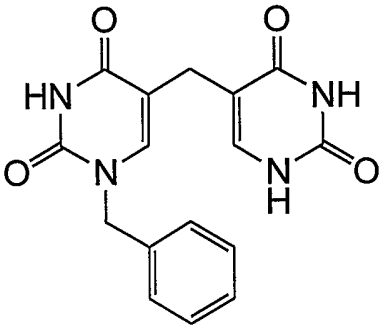
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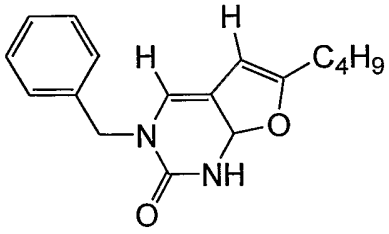
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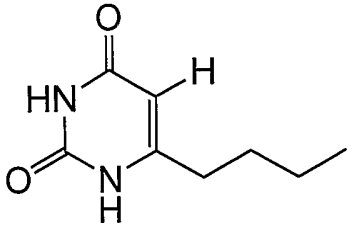
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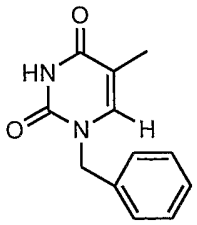
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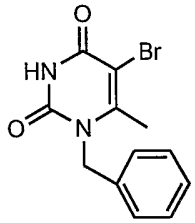
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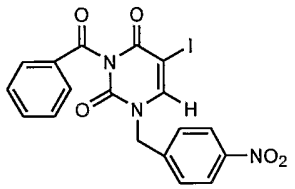
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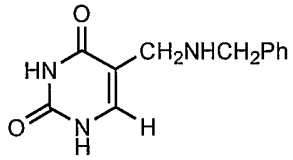


(77)



(78)

and



(79)

or acceptable salts or esters thereof.

15. The compound of any one of claims 1-15, wherein the acceptable salts are pharmaceutically acceptable salts.

16. A pharmaceutical composition comprising a sufficient amount of a compound of any one of claims 1-15, and a pharmaceutically acceptable carrier.

17. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition is useful for treating, preventing, or inhibiting a seizure-associated state.

18. The pharmaceutical composition of claim 17, wherein said seizure-associated state is epilepsy.

19. The pharmaceutical composition of claim 16, wherein said compound is anti-ictogenic.

20. The pharmaceutical composition of claim 16, wherein said compound is anti-epileptogenic.

21. A method of treating, preventing, or inhibiting a seizure-associated state in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of any one of claims 1-15, such that the seizure-associated state is treated, prevented, or inhibited.

22. A method of treating, preventing, or inhibiting ictogenesis in a subject comprising administering to a subject in need thereof a sufficient amount of a compound of any one of claims 1-15, such that ictogenesis is treated, prevented, or inhibited.

23. A method of treating, preventing, or inhibiting epileptogenesis in a subject comprising administering to a subject a sufficient amount of a compound of any one of claims 1-15, such that epileptogenesis is treated, prevented, or inhibited.

24. A kit for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a compound of any one of claims 1-15, and instructions for administering a sufficient amount of the compound to a subject, such that a seizure-associated state is treated, prevented, or inhibited.

25. A packaged pharmaceutical composition for treating, preventing, or inhibiting a seizure-associated state in a subject comprising a container holding a compound of any one of claims 1-15; and instructions for using the compound for treating, preventing, or inhibiting the seizure-associated state in a subject.

26. A kit for treating, preventing, or inhibiting ictogenesis in a subject comprising a compound of any one of claims 1-15, and instructions for administering a sufficient amount of the compound to a subject, such that ictogenesis is treated, prevented, or inhibited.

27. A packaged pharmaceutical composition for treating, preventing, or inhibiting ictogenesis in a subject comprising a container holding a compound of any one of claims 1-15; and instructions for using the compound for treating, preventing, or inhibiting ictogenesis in a subject.

28. A kit for treating, preventing, or inhibiting epileptogenesis in a subject comprising a compound of any one of claims 1-15, and instructions for administering a sufficient amount of the compound to a subject, such that epileptogenesis is treated, prevented, or inhibited.

29. A packaged pharmaceutical composition for treating, preventing, or inhibiting epileptogenesis in a subject comprising a container holding a compound of any one of claims 1-15; and instructions for using the compound for treating, preventing, or inhibiting epileptogenesis in a subject.

30. A method of treating, preventing, or inhibiting seizures in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of any one of claims 1-15, such that seizures are treated, prevented, or inhibited.

31 A method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from head trauma comprising administering to a subject a sufficient amount of a compound of any one of claims 1-15, such that epileptogenesis is treated, prevented, or inhibited.

32. A method of treating, preventing, or inhibiting seizures in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of any one of claims 1-15, such that seizures are treated, prevented, or inhibited.

33 A method of treating, preventing, or inhibiting epileptogenesis in a subject suffering from traumatic brain injury (TBI) comprising administering to a subject a sufficient amount of a compound of any one of claims 1-15, such that epileptogenesis is treated, prevented, or inhibited.

34. The method of claim 30 or 32, wherein the seizures are caused by epileptogenesis.

35. A method of preparing a compound of any one of claims 1-15 as described herein.