

SYNTHESIS OF TEMOZOLOMIDE AND ANALOGS

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FIELD OF THE INVENTION

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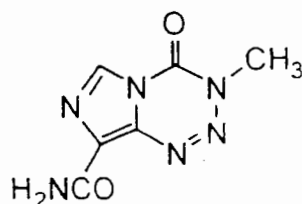
This invention relates to a novel process for the synthesis of temozolomide, an antitumor compound, and analogs thereof, and to intermediates useful in this novel process.

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BACKGROUND OF THE INVENTION

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Temozolomide, 3-methyl-8-aminocarbonyl-imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one, is a known antitumor drug; see for example Stevens *et al.*, *J. Med. Chem.* 1984, 27, 196-201, and Wang *et al.*, *J. Chem. Soc., Chem. Commun.*, 1994, 1687-1688. Temozolomide, the compound of formula 1:

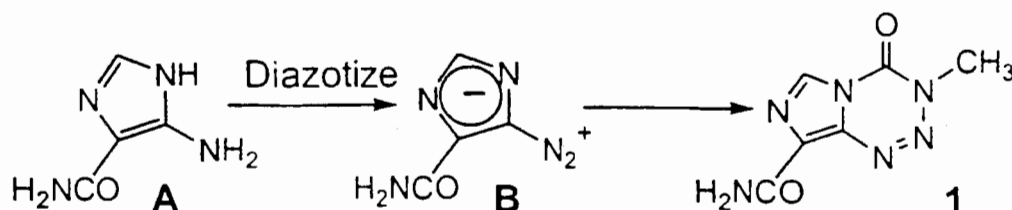


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is described in U.S. Patent No. 5,260,291 (Lunt *et al.*).

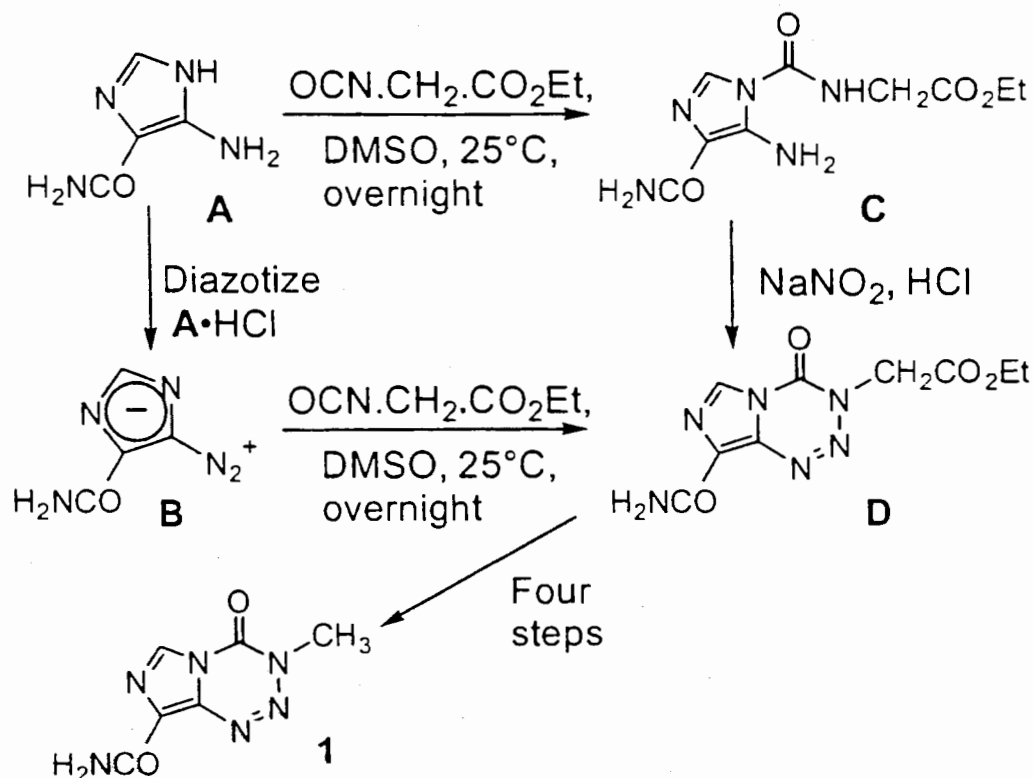
The synthesis of 1 by the process described in *J. Med. Chem.* 1984, 27, 196-201 is depicted in the scheme I below.

Scheme I:

In this process, 5-amino-1H-imidazole-4-carboxamide (A) is converted into 5-diazo-1H-imidazole-4-carboxamide (B), which is then cyclized with methylisocyanate in dichloromethane to provide a high yield of temozolomide. However, this process requires isolation of the unstable and potentially dangerous 5-diazo-1H-imidazole-4-carboxamide (B). Moreover, methylisocyanate is a difficult reagent to handle and ship, especially on the industrial scale, and indeed is better avoided in industrial manufacture. Furthermore, the cycloaddition of methylisocyanate requires a very long reaction time: Table I in *J. Med Chem.* 1984, 27,196-201, suggests 20 days. Additionally, Stevens *et al* mention that the cycloaddition of the methylisocyanate to the compound of the formula (B) can proceed through two different intermediates:

The production of I by the two processes described in *J. Chem. Soc., Chem. Commun.*, 1994, 1687-1688 provides a low overall yield from 5-amino-1H-imidazole-4-carboxamide (A): less than 20% (unoptimized - about 17% through 5-diazo-1H-imidazole-4-carboxamide (B) and about 15% through 5-amino-N¹-(ethoxycarbonylmethyl)-1H-imidazole-1,4-dicarboxamide (C)); Scheme II below

Scheme II:



Moreover, the unstable 5-diazo-1H-imidazole-4-carboxamide (B) still has to be isolated in the branch of this process that uses it as an intermediate.

Clearly, therefore, there is a need for synthetic methods that:

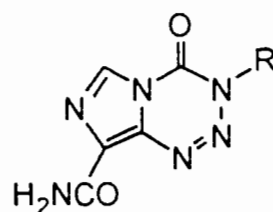
- are more convenient and higher yielding, especially on commercial scale;
- approach the synthesis of the temozolomide nucleus in novel ways;
- improve the preparation or use of intermediates for the processes.

SUMMARY OF THE INVENTION

The present invention provides processes for the preparation of temozolomide, alkyl analogs thereof and useful intermediates of temozolomide.

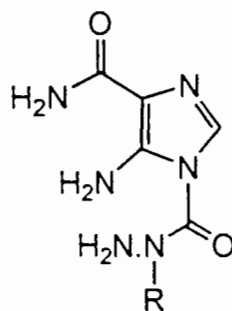
In one embodiment, this invention provides a process for the preparation of a compound of the formula IA:

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IA

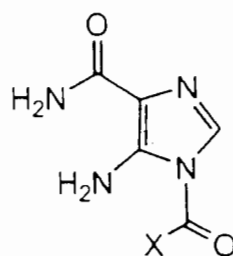
wherein R is an alkyl group having from 1 to 6 carbon atoms, the process comprising, reacting a compound of the formula II



II

with a suitable oxidation/cyclization agent in the presence of a soluble iodide, in an inert medium, under an inert atmosphere and at a temperature and for a time sufficient enough to produce a compound of the formula IA.

In another embodiment, this invention provides a process of preparing a compound of formula II by reacting a compound of the formula III:

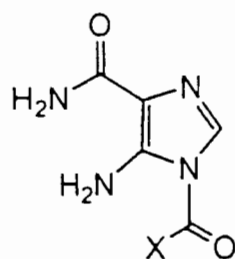


III

wherein X is a leaving group of the type that activates its adjacent carbonyl group towards nucleophiles, with a suitable hydrazine, i.e. R-NH-NH₂, wherein R is an alkyl group having 1 to 6 carbon atoms.

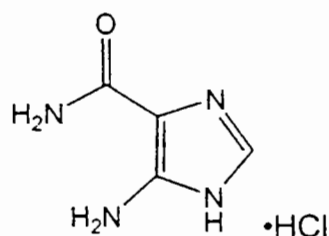
In another embodiment, this invention provides a process for preparing a compound of the formula III.

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III

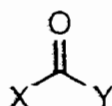
which comprises reacting a compound of the formula 4:



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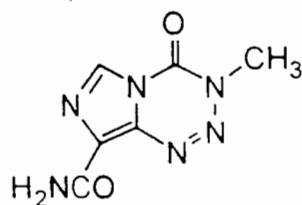
with a compound of the formula :



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wherein each of X and Y is the same or different leaving group, to yield compound III.

In yet another embodiment, this invention provides a novel process for preparing temozolomide (1):

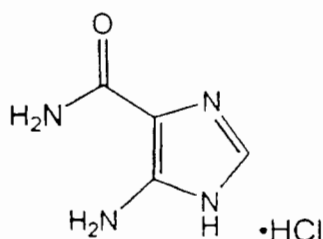


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comprising:

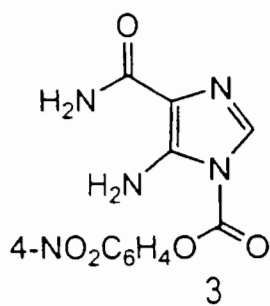
a) reacting compound 4:



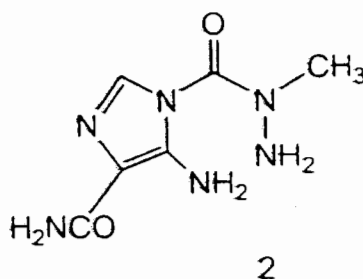
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with 4-nitrophenyl chloroformate in the presence of triethylamine in CH_2Cl_2 and under a nitrogen atmosphere at about 25°C to obtain compound 3:



b) reacting compound 3 with methylhydrazine in DMF at about 0°C to obtain compound 2:



and

c) reacting compound 2 with Bu_4NI in a 50/50 mixture of THF/ CH_3CN , at a temperature of about 60°C for about zero to sixty minutes, followed by the cooling of the reaction mixture to about 25°C and the addition of H_5IO_6 with stirring for about 10 to about 60 minutes to obtain temozolomide (1).

The present invention further provides various novel intermediates, specifically:

1-alkyl derivatives of 5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylic acid hydrazide wherein the alkyl group contains from 1 to 6 carbon atoms (i.e., alkyl derivatives at the N-1 hydrazine atom),

5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylic acid and active esters thereof, especially 4-nitrophenyl 5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylate, and

5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylic acid 1-methylhydrazide (which can alternatively be named as $\text{N}^1,5$ -diamino-N-methyl-1H-

imidazole-1,4-dicarboxamide).

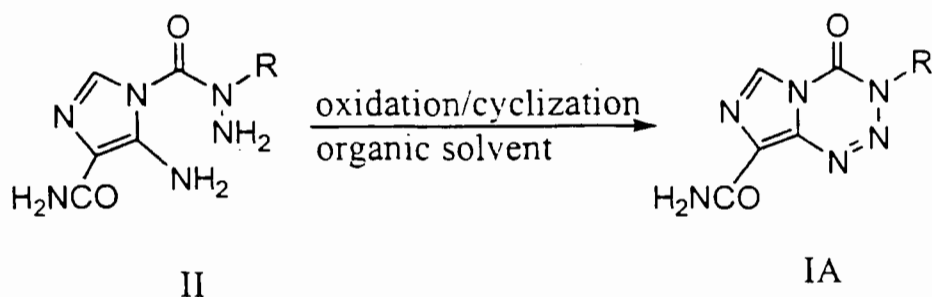
DETAILED DESCRIPTION

5 As used herein the following terms have the following meanings unless defined otherwise,

	DEC.HCl:	1-[3-(Dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride
10	DCC:	N,N'-Dicyclohexylcarbodiimide
	HOBT:	1-Hydroxybenzotriazole
	DMF:	N,N-Dimethylformamide
	EtOAc:	Ethyl acetate
	NMM:	4-Methyl morpholine
15	MeOH:	Methyl alcohol
	EtOH:	Ethyl alcohol
	Et ₂ O:	Diethyl ether
	BOC :	t-Boc or tert-Butyloxycarbonyl
	LiOH:	Lithium hydroxide
20	NaOH:	Sodium hydroxide
	KOH:	Potassium hydroxide
	acac	acetylacetonate
	DCC	dicyclohexylcarbodiimide
	NCS	N-chlorosuccinimide
25	NBS	N-bromosuccinimide

The term "alkyl" or "lower alkyl" means straight or branched alkyl chains of 1 to 6 carbon atoms.

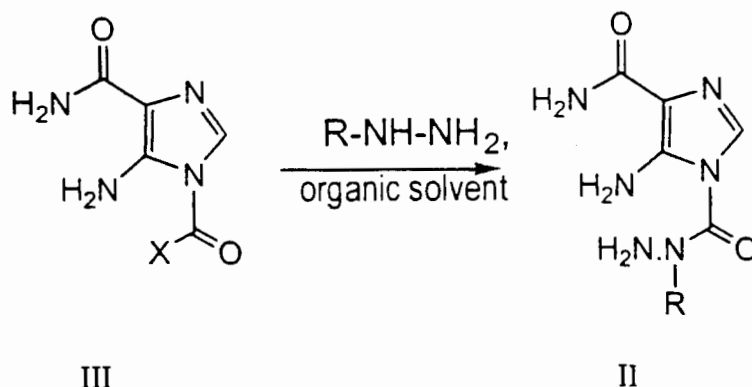
30 "R" is an alkyl chain that can be straight or branched, but is preferably straight, such as for example ,1-pentyl and 1-hexyl. Preferably, it is a lower alkyl group having 1 to 4 carbon atoms, such as 1-butyl, 1-methyl-propyl, 2-methyl-1-propyl, 1-propyl, 1-methyl-ethyl, ethyl or methyl, most preferably methyl.

Scheme III

The conversion of the compound of the formula II into the compound of the formula IA (Scheme III) requires concomitant or consecutive oxidation and cyclization to take place. The oxidizing/cyclizing agent may be, for example, periodic acid (H_5IO_6), iodine/potassium iodate, bromine or chlorine, or a reagent that oxidizes NH_2 to NZ , where Z represents Oxygen, (H, Hal), or Hal_2 , wherein Hal is halogen and wherein the halogen is chlorine, bromine or iodine. Other suitable oxidizing agents include KI/KIO_3 , I_2 , I_2/KIO_3 , ICl , ICl_3 , I_2O_5 , $\text{NCS/Me}_2\text{S}$, $\text{NBS/Me}_2\text{S}$, $\text{DCC/DMSO/H}_3\text{PO}_4$, peracetic acid, $\text{VO}(\text{acac})_2/\text{O}_2$, $\text{VO}(\text{acac})_2/\text{t-BuOOH}$, V_2O_5 , $\text{Bu}_4\text{NI/O}_2$, and MnO_2 . Preferably, the oxidizing agent is H_5IO_6 and the reaction is preformed in the presence of an iodide that is soluble in the reaction medium, the medium being an inert organic solvent. A quaternary ammonium iodide is typically preferred, and examples include tetraalkylammonium iodides such as Bu_4NI . However, since the iodide functions catalytically, a small amount of an inorganic iodide, even if only sparingly soluble in the reaction medium, can also be used, i.e. KI .

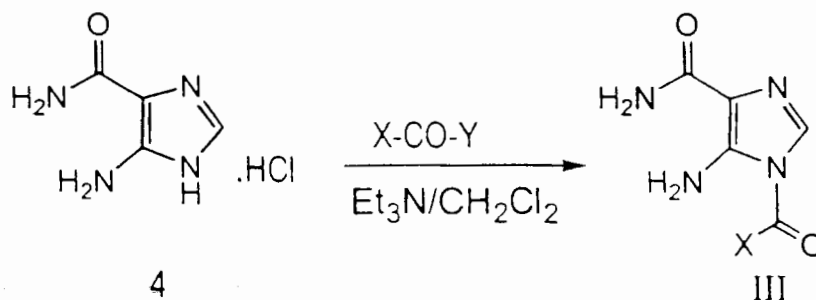
The process of scheme III is carried out in an inert organic solvent that is selected from a non-nucleophilic solvent such as DMF, an ether, such as t-butyl-methyl-ether, a cyclic ether, such as THF or dioxane, acetonitrile, methylene chloride, toluene, an alkyl alkanoate wherein the alkyl group has 1 to 4 carbon atoms and the alkanoate group has 2 to 4 carbon atoms, i.e. ethyl acetate, and mixtures thereof. The reaction is preferably carried out under an inert atmosphere, e.g., under nitrogen, and at a suitable temperature range from about -20°C to about $+70^\circ\text{C}$, preferably about 0°C to about $+60^\circ\text{C}$.

Preferably, the organic solvent is a 50/50 mixture of THF/acetonitrile and since the compound IA is basic, no further acid-binding agent is normally necessary.

Scheme IV

In the preparation of a compound of formula II shown in Scheme IV, compound III is reacted with an alkylhydrazine of the formula R-NH-NH₂. R is a lower alkyl as defined above and X is a leaving group of the type that activates its adjacent carbonyl group towards nucleophiles. X may be, for example, an active esterifying group such as a phenyloxy or 2-naphthyloxy group, or a substituted phenyloxy group wherein the substituents are electron withdrawing, e.g., a 2- or especially 4-nitro group, or a pentafluoro group. X may also be, for example, chlorine, bromine or iodine.

The reaction is conducted in an inert organic solvent such as, for example, DMF, THF, CH₂Cl₂, acetonitrile or mixtures thereof, under an inert atmosphere and at a temperature of about 0°C. Preferably the reaction is conducted under a nitrogen atmosphere, at a temperature of 0°C using DMF as the solvent.

Scheme V

In the preparation of compound III shown in scheme V above, compound 4 is reacted with a compound of the formula:

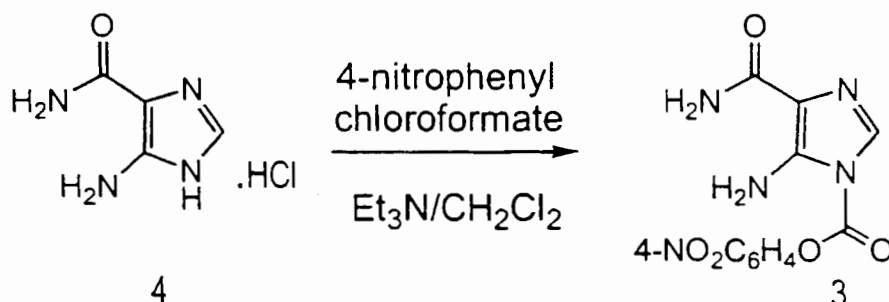
X-CO-Y

wherein X and Y are the same or different leaving group. X or Y can be for example, halogen, phenyloxy, 2-naphthyloxy or a substituted phenyloxy group, wherein said substituents on said phenyloxy groups are, for example, nitro, pentafluoro, chlorine, bromine, iodine or combinations thereof. Preferably, X and Y are different i.e. Y is halogen and X is a phenyloxy, 2-naphthyloxy or a substituted phenyloxy group. More preferably, Y is a halogen such as, for example, bromine, chlorine, or iodine with chlorine being most preferred and X is a phenyloxy, 2-naphthyloxy or a substituted phenyloxy group. Yet even more preferred, Y is chlorine and X is a substituted phenyloxy group with said substituents on said phenyloxy groups being nitro or pentafluoro. Still even more preferred, Y is chlorine and X is nitro-phenyloxy. Examples of compounds of the formula X-CO-Y include, but are not limited to, chloroformate and bromoformate esters of reactive leaving groups such as for example 4-nitrophenyl chloroformate.

The reaction is conducted in the presence of an organic or inorganic acid-binding agent such as, for example, a tertiary amine i.e. pyridine, 2,6-lutidine and triethylamine or a base such as, for example, sodium and potassium bicarbonates or carbonates, with triethylamine being the most preferred.

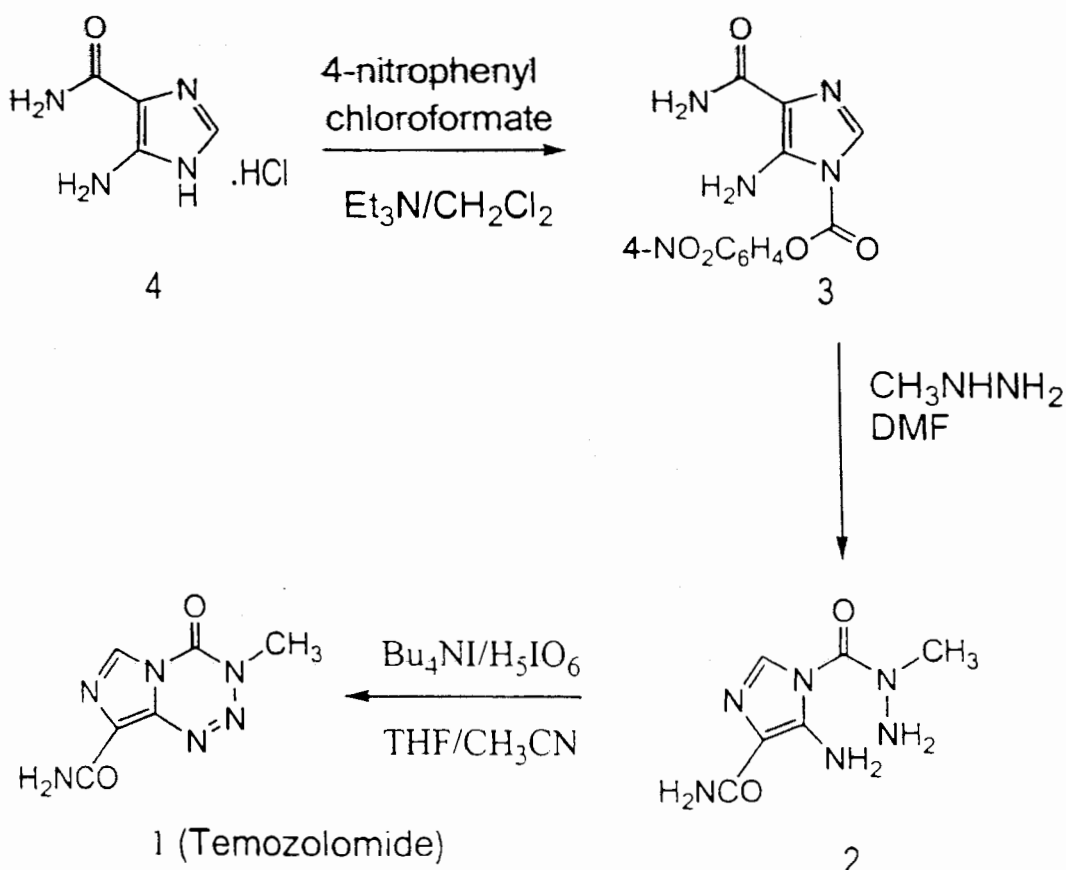
An organic solvent, preferably an inert organic solvent such as, DMF, THF, CH_2Cl_2 , acetonitrile, and ethyl acetate is used, with CH_2Cl_2 being preferred. The reaction is conducted under an inert atmosphere e.g. a nitrogen atmosphere and at a temperature range of about -20°C to about $+50^\circ\text{C}$.

In a preferred embodiment of this reaction, 5-amino-1H-imidazole-4-carboxamide•HCl (4) is allowed to react with 4-nitrophenyl chloroformate under a nitrogen atmosphere in the presence of triethylamine in CH_2Cl_2 to obtain compound 3 (Scheme VI).

Scheme VI

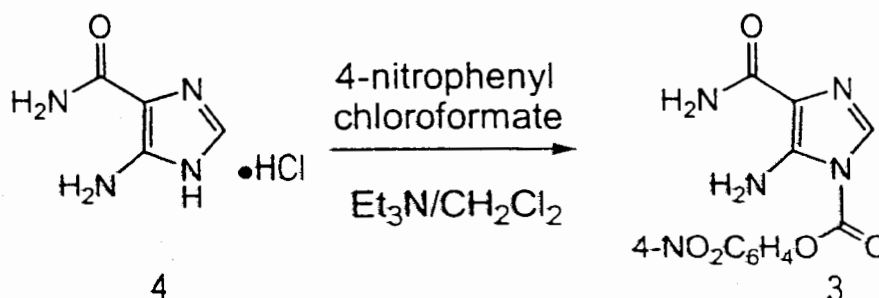
Thus, the present invention provides a novel synthesis for Temozolomide and lower alkyl analogs thereof, which proceeds in three simple steps from a commercially available starting material, 5-amino-1H-imidazole-4-carboxamide•HCl and avoids the use of hazardous materials such as the unstable 5-diazo-1 H-imidazole-4-carboxamide and methyl- isocyanate.

A preferred embodiment of the process, directed to the preparation of temozolomide itself, is shown in Scheme VII:

Scheme VII:

EXAMPLES

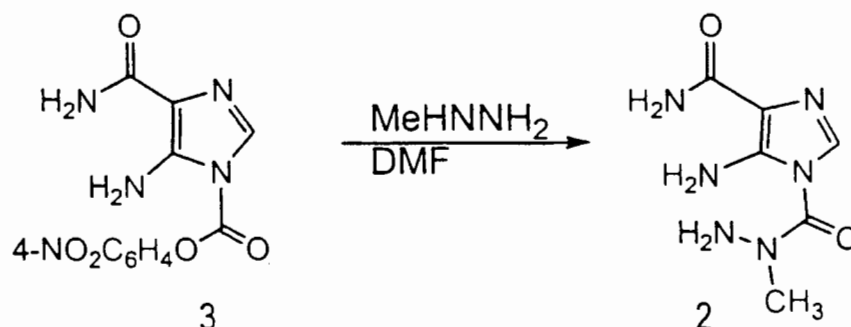
The following Examples illustrate but do not in any way limit the present invention. Chemicals obtained from Aldrich Chemical Company (Milwaukee, WI) are identified by their catalog number. It should be noted that nomenclature may differ slightly between this specification and the Aldrich catalog.

EXAMPLE 1 Preparation of Temozolomide (1)Step A Preparation compound (3)

5-Amino-1H-imidazole-4-carboxamide•HCl (4) (25 g, 0.154 mol) (Aldrich 16,496-8), CH₂Cl₂ (0.6 L) and Et₃N (45 mL) (Aldrich, 13,206-3) were placed into a dry 2-liter, three-necked flask equipped with dropping funnel, a gas inlet tube, a gas outlet tube, reflux condenser and mechanical stirrer, and maintained under a positive pressure of nitrogen at ambient temperature. The mixture was stirred, and a solution of 400 mL of 4-nitrophenyl chloroformate (34 g, 0.169 mol) (Aldrich, 16,021-0) in CH₂Cl₂ was added dropwise. The reaction mixture was stirred vigorously for 4 hours and then left to stand for 18 hours at room temperature. The precipitate was collected by vacuum filtration and washed with H₂O (1.5 L) to afford the product (3) as a pale yellow solid (42 g, 0.144 mol).

¹H NMR (400MHz, DMSO-d₆, δ): 8.40 (d, 2H), 7.83 (s, 1H), 7.74 (d, 2H), 7.08 (bs, 1H), 6.95 (bs, 1H), 6.52 (s, 2H).

Step B Preparation of compound (2)



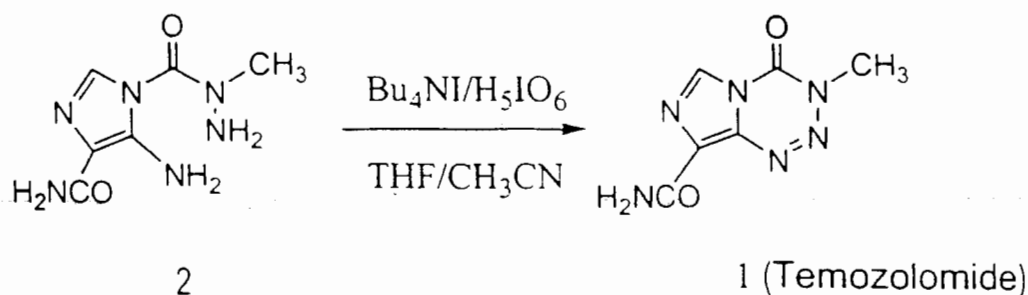
Compound (3) (42 g, 0.144 mol) and DMF (0.27 L) were placed into a dry 1-liter, three-necked flask equipped with dropping funnel, a gas inlet tube, a gas outlet tube, reflux condenser and mechanical stirrer, and maintained under a positive pressure of nitrogen. The reaction mixture was cooled to 0°C, and methylhydrazine (10 mL, 0.188 mol) (Aldrich, M5,000-1) was added dropwise. The reaction mixture was stirred vigorously for 1 hour at 0°C and was then poured into EtOAc (2.1 L). The precipitate was collected by vacuum filtration and was dried under vacuum (20 mm Hg, room temperature, 18 hours) to afford (2) as a tan solid (27.1 g, 0.137 mol).

¹H NMR (400MHz, DMSO-d₆, δ): 7.62 (s, 1H), 6.85 (bs, 1H), 6.75 (bs, 1H), 6.00 (s, 2H), 5.10 (s, 2H), 3.15, s, 3H). mp: 188⁰C (dec.).

Analysis: Calcd for $C_6H_{10}N_6O_2$: C, 36.36; H, 5.09; N, 42.41.

Found: C, 36.46; H, 4.99; N, 42.12.

Step C Preparation of Temozolomide (1)



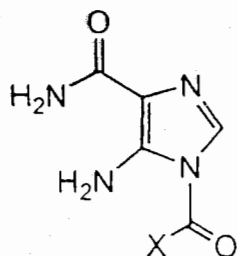
Compound (2) (500 mg, 2.5 mmol), Bu₄NI (95 mg, 0.25 mmol), THF (250 mL) and CH₃CN (250 mL) were placed into a dry 1-liter, three-necked flask

equipped with dropping funnel, a gas inlet tube, a gas outlet tube, reflux condenser and mechanical stirrer, and maintained under a positive pressure of nitrogen. The reaction mixture was heated at 60°C for 20 mm and then cooled to room temperature. H₅IO₆ (1.14 g, 5 mmol) was added and the reaction mixture was stirred vigorously at room temperature for 1 hour. The resulting solution was treated with saturated aqueous Na₂S₂O₃ (5 mL) and was then concentrated under reduced pressure to dryness. The residue was treated with CH₃CN (200 mL) and was filtered. The filtrate was concentrated and chromatographed on a column of silica gel (1.5% to 2% AcOH/EtOAc) to afford temozolomide (1) (280 mg).
¹H NMR (400MHz, DMSO-d₆, δ): 8.80 (s, 1H), 7.80 (bs, 1H), 7.66 (bs, 1H), 3.43 (s, 3H).

While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

CLAIMS

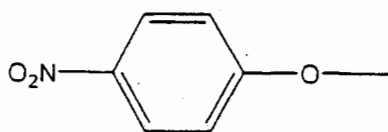
1. A compound of the formula:



III

or an active ester thereof, wherein X is a leaving group of the type that activates its adjacent carbonyl group towards nucleophiles.

2. The compound of claim 1 wherein X is OH, said compound being 5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylic acid.
3. The compound of claim 1 wherein X is



said compound being 1-(4-nitrophenyl)-5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylate.

4. The compound of claim 1 wherein X is -N(CH₃)-NH₂, said compound being 5-amino-4-(aminocarbonyl)-1H-imidazole-1-carboxylic acid-1-methylhydrazide.