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AN ALTERNATE AND SCALABLE PROCESS FOR THE SYNTHESIS OF TEMOZOLOMIDE

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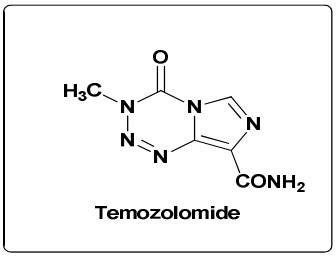
Abstract:
An alternate and scalable process for the synthesis of anti-cancer drug temozolomide is described. Synthesis of urea intermediates is disclosed with high yields and purity using an alternate carbamate reagent which would avoid the usage of toxic methyl isocyanate. Optimum and robust process for the conversion of urea intermediate to temozolomide is presented.

Keywords:
Temozolomide, urea derivative, anti-cancer, 4-nitrophenyl methylcarbamate, diazotization.

Introduction

Temozolomide[I] is an oral chemotherapy drug. It is an alkylating agent used as a treatment of some brain cancers; as a second-line treatment for astrocytoma and a first-line treatment for glioblastomamultiforme. The anti-tumor drug temozolomide has shown useful clinical activity against brain tumours[II] and metastatic melanoma, two malignancies hitherto considered poorly treatable.It is sold in the US market as hard capsules containing 5 mg, 20 mg, 100 mg or 250 mg as temodar by Schering Corporation.

There are various methods reported for the synthesis of temozolomide, in most of the methods, methyl isocyanate is used directly or in situ generation[III]. The original synthesis of temozolomide[IV]discaribed from 5-diazoimidazole-4-carboxamide which intern prepared from 5-amino-1H-imodazolo-4-carboxamide. 5-diazo-1H-imidazole-4-carboxamide is reacted with methylisocynate in dichloromethane to form in situ urea intermediate with concomitant cyclisationto yield temozolomide**(1)**. However, methyl isocyanate is noxious and the usage of large quantities in the commercial synthesis is extremely difficult.

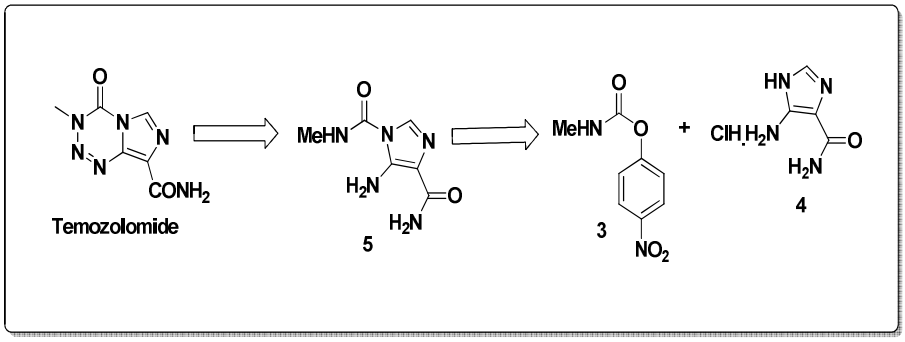


Shen-Chun Kuo et.al.[V] described the synthesis of temozolomide by cyclization of 5-amino-l-(N-rnethyl-hydrazinocarbonyl)-lH-imidazole-4-carboxylic acid in the presence of tetra butyl nickel and periodic acid to form a reaction mixture. Use of time consuming and cumbersome column chromatography for isolation of product makes the process not suitable to employ at industrial scale. The same group disclosed a process for the preparation of temozolomide from protected imidazole intermediate in which l-methyl-3-carbamoyliminomethyl-urea reacts with *N*- protected amino cyano acetamide in the presence of acetic acid in a suitable solvent to form an *N*- protected imidazole intermediate which is then cyclized in the presence of lithium chloride to minimize undesired cyclisation product. After cyclisation, the protected group has to be removed which makes the process more laborious with more number of steps. Based on the above mentioned methods it is very clear that there is a need develop a process which would be expedient for commercial scale up. Herein, we report an improved and scalable synthesis of temozolomide from commercially available starting materials.

RESULTS AND DISCUSSION

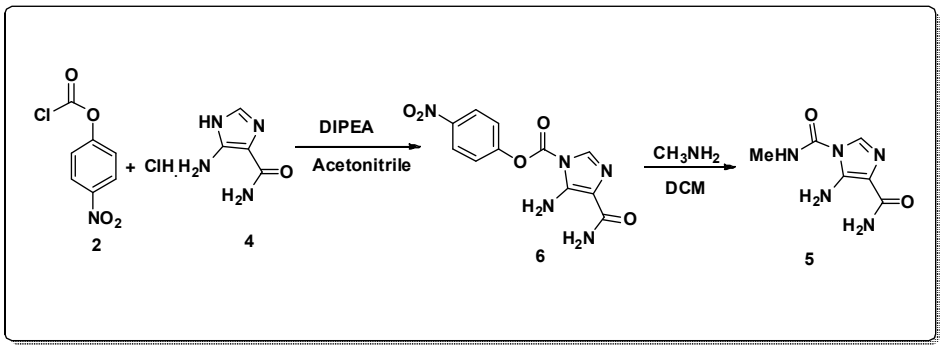
In formulating the synthetic plan, we assessed the usage of non-toxic carbamate **(3)** and envisioned that **1** could be synthesized *via* the coupling between carbamate **(3)** and imidazole carboxamide intermediate **4** to furnish compound **5** followed by diazotization and in situ cyclisation (**Scheme-1**).

Scheme-1: Retro synthetic analysis:



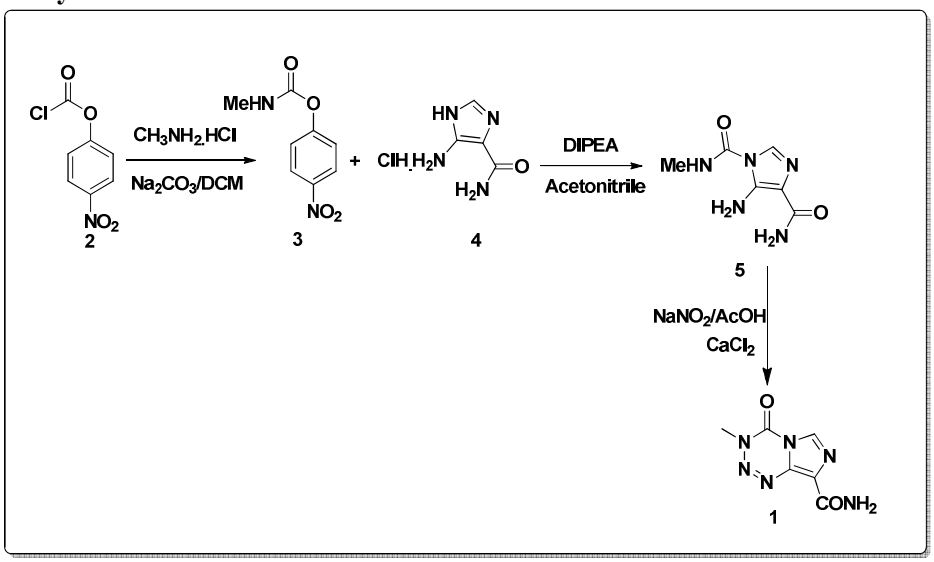
Initially, we adopted a known route to prepare urea intermediate **5** (scheme-2), in this method we were able to prepare intermediate **6** in good yields with consistency. However during the conversion of carbamate intermediate **6** to urea intermediate **5**, the results are inconsistent and sometimes product formation is not observed. As imidazole also acts as a leaving group in intermediate **6** like CDI, there is a possibility of formation of dimethyl urea impurity from int-5 in the presence of methylamine in parallel.

Scheme-2: Synthesis of Urea intermediate (5):



The present synthesis of temozolomidebegan from readily available 4-nitrophenylchloroformate and methyl amine hydrochloride (Scheme-3). To circumvent the difficulties, we planned to use carbamate intermediate **3**[VI] for the synthesis of urea intermediate **5**.

Scheme-3: **Synthesis of Temozolomide:**



The synthesis of carbamate (**3)** tried under different catalytic conditions and the results were summarized in **Table-1**. Initially, methyl amine hydrochloride treated with nitrophenylchloroformate in the presence of triethylamine as base and observed the desired product formation, however, isolated yield is low and triethylamine hydrochloride byproduct could not be removed completely from the product. Surprisingly, when the reaction carried out with triethylamine as base in acetonitrile, desired product formation is not observed.

Table-1: Optimization of carbamate formation reaction with methylamine:

Entry	2 (eq)	Methylamine HCl (eq)	Reaction condition	Yield (%)
1	1.0	1.2	Na_2CO_3 (3.0 eq), DCM,RT, 20h.	60%
2	1.0	1.2	TEA(2.2 eq), DCM,RT, 16h.	30%
3	1.0	1.2	K_2CO_3 (2.0 eq), DCM,RT, 16h.	48%
4	1.0	1.2	TEA(3.0 eq), acetonitrile, RT, 16h.	-

Moderate yields were obtained when the reaction carried out in the presence of potassium carbonate as base. Alternately, when sodium carbonate used as base, the reaction proceeded smoothly at room temperature and simple filtration of salts followed by evaporation of filtrate afforded the product in pure form.

Few conditions tried for the synthesis of int **5**[VII] and the results captured in **Table-2.** The urea formation step tried on intermediate **4**[VIII] in the presence of triethylamine and diisopropylethylamine.

Table-2: Optimization of urea formation reaction with 4-nitrophenylmethylcarbamate:

Entry	3 (eq.)	Reaction condition	Yield (%)
1	1.94	DIPEA (1.55 eq), ACN, RT, 24 h	95%
2	2.0	TEA (1.55 eq), ACN, RT.	-
3	2.0	DIPEA (1.55 eq), DCM, RT.	-

Though the desired product formation is seen in all these conditions, complete conversion of starting material is not observed when triethylamine used as base even after conducting the reactions for longer hours (>36h). Finally, DIPEA in acetonitrile condition proved to be better in terms of conversion. After completion of the reaction, simple filtration of product followed by slurry in acetonitrile provided urea intermediate **5** with excellent yield and purity. The two step process provided a simplified and practical access to the key intermediate **5** in large quantities utilizing inexpensive raw materials and easy isolation of product as solid.

There are several methods reported for the final diazotization followed by cyclisation to obtain Temozolomide[IX]. We have selected few best conditions for investigation; aqueous and

non-aqueous conditions studied for diazotization followed by cyclisation. Observed desired product formation in aqueous conditions, remarkably desired product formation was not observed in non-aqueous conditions.

Table-3: Optimization of diazotization followed by cyclisation:

Entry	Reaction condition	Yield (%)
1	Aq. $NaNO_2$, AcOH, $CaCl_2$	28.6
2	Aq. $NaNO_2$, AcOH, LiBr ($Na_2S_2O_4$)	23.0
3	Aq. $NaNO_2$, AcOH, LiCl ($Na_2S_2O_4$)	25.0
4	Aq. $NaNO_2$, AcOH, $CaCl_2$ (NaCl saturation of aqueous layer)	29.6
5	Aq. $NaNO_2$, (L)-Tartaricacid, $CaCl_2$	10
6	Isoamyl nitrite	-

Though non-aqueous conditions are not reported in the literature for the diazotization & cyclisation step (for Temozolomide), to simplify the work up and improve the yields, final step attempted under isoamyl nitrite in acetonitrile and acetic acid conditions.However the desired product formation is not observed. The diazotization followed by cyclisation tried under aqueous sodium nitrite and acetic acid in the presence of additives like $CaCl_2$, LiCl and LiBr conditions. In all the cases desired product formation is observed and isolated yields arecomparable. Based on the cost, $CaCl_2$ is suitable for commercial synthesis. Poor yields obtained when the reaction conducted in the presence of tartaric acid.

After completion of the reaction, direct filtration and purification tried under variety of conditions but the pure Temozolomide could not be isolated. So, after the reaction, reaction mass extracted with 2.5% DMSO:DCM conditions; evaporation of DCM and filtration of precipitated solid and washing with ethylacetate, drying to provide Temozolomide with >99% HPLC purity.

The present process overcomes the major disadvantages of previously reported methods by avoiding the usage of hazardous methyl isocyanate and also involving easy isolation of Temozolomide either by filtration or by using less amount of the solvent.

Experimental Section

General information

Solvents and reagents were obtained from commercial sources and used without further purification. ^{1}H NMR spectra were recorded in $CDCl_3$, DMSO-d_6 at room temperature on a Varian Mercury spectrometer plus 400 MHz using TMS as an internal standard. ^{13}C NMR spectra were obtained from a Varian Mercury plus 100 MHz spectrometer in DMSO-$d_6$at room temperature. The mass spectrum (70 eV) was recorded on an HP 5989 A LC−MS spectrometer. TLC analyses were performed on Mercksilica gel 60F254 plates.

General procedure for the synthesis of 4-nitrophenyl methylcarbamate (3):

To a stirred solution of methyl amine hydrochloride (20g, 0.296 mol) in dichloromethane (250 mL) was added sodium carbonate (79.0 g, 0.745 mol) at 20-25^{o}C. The suspension was

stirred for 10 min and 4-nitrophenyl chloroformate **(2)**(50g, 0.248 mol) in dichloromethane (250 mL) was added slowly at 20-25^{o}C. The reaction mixture was stirred at room temperature for 24h. After consumption of starting material, the reaction mixture was filtered and cake washed with dichloromethane (50 mL). The wet cake was slurred in 200 mL dichloromethane, filtered and washed with dichloromethane (50 mL). The combined filtrate was concentrated under reduced pressure to obtain 4-nitrophenyl methylcarbamate **(3)**(30.9 g, 65%) as light yellow solid. The crude material used in the next step without further purification.

^{1}H NMR ($CDCl_3$, 400 MHz):δ 8.24 (d, J = 7.6 Hz, 2H), 7.31 (d, J = 7.6 Hz, 2H), 5.24 (bs, 1H), 2.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz):δ 155.94, 153.88, 144.63, 125.03, 121.92, 27.67.

General procedure for the synthesis of 5-amino-N1-methyl-1H-imidazole-1,4-dicarboxamide (5):

To a stirred suspension of 5-amino-1H-imidazole-4-carboxamide hydrochloride (**4)** (10g, 0.061 mol) in acetonitrile (80 mL) was added diisopropylethylamine [DIPEA (12g, 0.095 mol)] at room temperature. The reaction mixture was stirred at room temperature for 10min followed by 4-nitrophenyl methylcarbamate**(3)**(25g, 0.127 mol) was added. The reaction mixture was stirred at room temperature for 24h. After consumption of starting material, the reaction mixture was filtered and the cake washed with acetonitrile (20 mL). The obtained solid was slurred in acetonitrile (80 mL), filtered and cake washed with acetonitrile (20 mL). The obtained solid dried at room temperature for 24h to afford 5-amino-N1-methyl-1H-imidazole-1, 4-dicarboxamide **(5)**(10 g, 89.3%) as an off white solid.

^{1}HNMR (DMSO-d_6, 400 MHz): δ 8.41 (q, 1H), 7.59 (s, 1H), 6.86 (bs, 1H), 6.77 (bs, 1H), 6.35 (s, 2H), 2.78 (d, J = 3.2 Hz, 3H);^{13}CNMR (DMSO-d_6, 100 MHz): δ 166.37, 150.72, 143.48, 126.14, 111.24, 26.59; Mass (*m/z*): 183.9 (M+); HPLC purity: 98.13%.

General procedure for the synthesis of (3-methyl-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5]tetrazine-8 carboxamide) temozolomide (1):

To a stirred suspension of 5-amino-N 1-methyl-1H-imidazole- 1, 4- dicarboxamide**(5)** (5g, 0.027 mol) and acetic acid (4.5 mL) was added sodium nitrite (2.5g, 0.036 mol) in water (5.0 L) at -5 to 0^{o}C at a rate so that temperature does not rise above 0-5^{o}C. The reaction mixture was stirred at 0-5^{o}C for 2h. After consumption of starting material, ice bath was removed and calcium chloride (12.5 g) was added to the reaction mixture and stirred at room temperature for another two hours. The reaction mass was extracted with a 2.5% solution of dimethyl sulfoxide in dichloromethane (5 x 500 mL). Combined organic layer was dried over sodium sulfate and the solvent was distilled below 40^{o}C. After removing the dichloromethane completely, the dimethyl sulfoxide suspension was cooled to 10-20^{o}C and filtered the solid. The solid was washed with ethyl acetate (15 mL) and dried at room temperature. The obtained solid was slurried in ethyl acetate (40 mL) and heated at 40-45^{o}C for 1h. The solid was filtered and dried at room temperature to yield temozolomide**(1)** (1.5g, 28.6%) as an off white solid.

^{1}HNMR (DMSO-d_6, 500 MHz): δ 8.81 (s, 1H), 7.78 (bs, 1H), 7.66 (bs, 1H), 3.86 (s, 3H); ^{13}CNMR (DMSO-d_6, 100 MHz): δ161.49, 139.15, 134.56, 130.50, 128.33, 36.11; Mass (*m/z*): 194.8 (M+).HPLC purity: 99.61%.

Conclusion

In conclusion, an alternate and scalable process for the synthesis of anti-cancer drug, Temozolomide has been described. The key urea intermediate has been successfully synthesized with high yields and purity using an alternate reagent which avoided the usage of toxic methyl isocyanate. Optimum and robust process for the final step (conversion of urea intermediate to Temozolomide) has been disclosed.

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