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Synthesis and characterisation of Aryloxy derivative of Bis-Azetinone for Anticancer Activity

Pramila. T,
Associate Professor, Bharathi College of Pharmacy,Bharathi Nagar, Maddur Taluk, Mandy District,Karnataka.

Abstract

Several pharmacological activities like anticancer, antimicrobial, antibacterial, antifungal and antiviral activity have been seen in the case of bis azitadione. Azitadione moiety is present in the β lactam antibiotics. The above observation promoted us to synthesize some bridged azitadione such as bis azitadione and its derivative for anticancer activity. A series of novel aryloxy bis azitadione have been synthesized by the reaction with benzocaine as a starting compound which undergo nucleophillic addition reaction with phenyl hydrazine hydrate that on treatment with palladium catalyst on reduction that on treatment with different substituted phenoxy acetyl chloride gives aryloxy bis azitadione derivatives (A_1 –A_{10}). The structures of the synthesized derivatives were confirmed by IR, H^1-NMR, Mass spectra. The synthesized compounds were screened for the *invitro* anticancer activity. The anticancer activity data of the synthesized compound were found to be potent.

Keywords: Bis azitadione, Benzocaine, *Invitro* anticancer activity.

INTRODUCTION

Bis-Azetidione derivative [1] having varied biological features are still of great scientific interest. They are widely found in bio-organic and medicinal chemistry due to its applications in drug discovery and have reported to possess a wide spectrum of various biological properties such as anti- cancer[2-3], anti-fungal[3,4], antimicrobial[4,5], antibacterial[5] and anti-viral[6] activity. Also Azetidione nucleus is associated in variety of biological activities. Being a heterocyclic compound, Beta-Lactam is found as a starting material for the synthesis of larger number of bioactive pharmacophores. Its tatuomeric forms makes it relatively stable, although as a heterocyclic it has reactive sites which allows for functionalization.

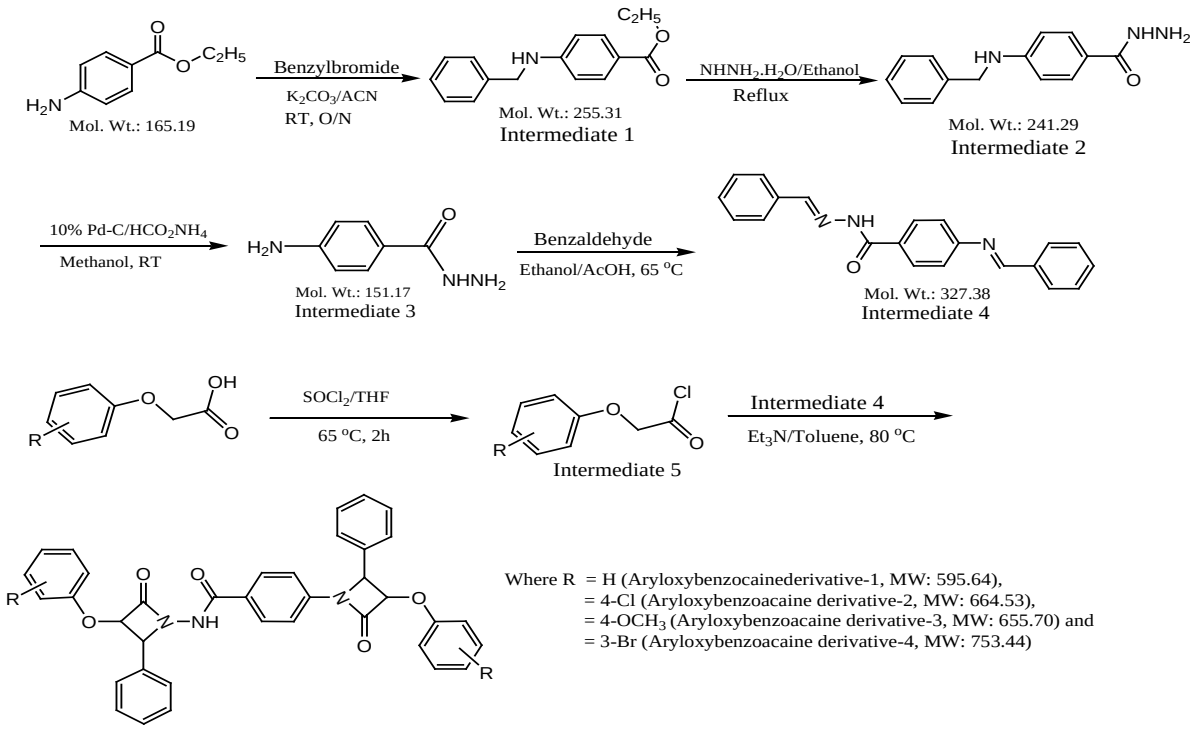
The activity of Azetidione occupies a prominent place in the field of anti cancer agents. With the view of above facts we have planned our work to synthesize and to develop its different Azetidione derivatives.

MATERIAL AND METHOD

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on SHIMADZU FT-IR 8400 with KBr pellets. H^1-NMR spectra were recorded on VNMRS-400 *agilent-NMR*. The chemical shifts are reported as ppm down fields from TMS. Mass spectra were recorded on LCMS. The purity of the compound was checked by TLC on pre-coated SiO_2 gel (604 GF 254) aluminium plates (E Merck)

1. SYNTHESIS OF ARYLOXYBENZOCAINE DERIVATIVES

The synthesis of aryloxy benzocaine derivatives were synthesized by following the General Procedure:



Where R = H (Aryloxybenzocainederivative-1, MW: 595.64),
= 4-Cl (Aryloxybenzoacaine derivative-2, MW: 664.53),
= 4-OCH_3 (Aryloxybenzoacaine derivative-3, MW: 655.70) and
= 3-Br (Aryloxybenzoacaine derivative-4, MW: 753.44)

Intermediate 1: Synthesis of Ethyl 4-(benzyl amino) benzoate

To a 100 mL RBF charged with acetonitrile(50.0 mL), ethyl 4-aminobenzoate (5.0 g, 0.03 mole) and benzyl bromide (5.7 g, 0.033 mole), to the stirred reaction mixture was added K2CO3 (8.37 g, 0.06 mole) at room temperature under nitrogen atmosphere. After the addition, the reaction mixture was kept for stirring at room temperature. After completion of the reaction (monitored by TLC/LCMS), the solvent was removed under vacuo, extracted with ethyl acetate. The combined organic layer was washed with water, drier over anhydrous Na2SO4 and concentrated to obtain the crude product, which was further purified by column chromatography to obtain the title product (3.5 g). LC MS: 254.1 (M - H).

Intermediate 2: Synthesis of 4-(benzylamino) benzohydrazide

To a 100 mL RBF charged with ethanol (30.0 mL) and ethyl 4-(benzylamino) benzoate (2.5 g, 0.0098 moles).To the stirred reaction mixture was added hydrazine hydrate (0.74 g, 0.014 moles) under nitrogen atmosphere and heated at reflux temperature. After completion of the reaction (monitored by TLC/LCMS), the reaction mixture was concentrated under vacuo, the residue was extracted with ethyl acetate. The combined organic layer was washed with water, drier over anhydrous Na2SO4 and concentrated to obtain crude product, which was purified by re-crystallization with diethyl ether to obtain the title product (1.2 g). LC MS: 239 (M -2H).

Intermediate 3: Synthesis of 4-aminobenzohydrazide

To a stirred solution of 4-(benzylamino)benzohydrazide (1.0 g, 0.004 mole) in 10.0 mL of methanol(10 mL), was added ammonium formate (2.5 g, 0.04 mole) followed by 10% palladium carbon (0.2 g, 20 mole%)) under nitrogen atmosphere. Then, the reaction mixture stirred at room temperature until completion. The reaction mixture was filter through celite bed washed with methanol. The combined washings concentrated under reduced pressure to obtain the title product, triturated with ether and n-hexane to obtain the title product (0.6 g). **LC MS**: 151.7(M + H).

Intermediate 4: Synthesis (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide

To a stirred solution of 4-(benzylamino) benzohydrazide (1.0 g, 0.0066 moles) in 10.0 mL of absolute ethanol was added benzaldehyde (1.4 g, 0.013 moles) and acetic acid (1.0 mL) at room temperature. The reaction mixture was heated at 80 ^{o}C in presence of nitrogen atmosphere about 4 hours. After completion, the reaction mixture was concentrated. The obtained residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified chromatography using ethyl acetate and n-hexane as an eluting system to obtain the title product (0.6 g) **LC MS**: 328.0 (M +H).

Intermediate 5: Synthesis of phenoxy acetyl chloride

To a 50 mL RBF charged with phenoxy acetic acid (0.5 g, 0.0036 mole) and THF (10 mL), was added thionyl chloride (0.65 g, 0.0054 mole) under cooling. The reaction mixture was heated at 65 ^{o}C, and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g) as such which was taken for next step.

Aryloxy benzocaine derivative-1: N, 4-bis (2-oxo-3-phenoxy-4-phenylazetidin-1-yl) benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylidene amino) benzohydrazide (0.52 g, 0.0016 mole), triethylamine (0.32 g, 0.0064 mole) and toluene (5 mL). The reaction mixture was heated at 80 ^{o}C, was added phenoxy acetyl chloride (0.5 g, 0.0032 mole) in toluene (5 mL) under nitrogen atmosphere. To reaction mixture was heated for 3 hours, after completion of the reaction, the reaction mixture was concentrated. The obtained residue was neutralized with 10% $NaHCO_3$ solution, extracted with ethyl acetate. The combined extract was washed with water dried over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.03 g), **LC MS:** 596.3 (M+H).

Intermediate 6: Synthesis of 4-chloro phenoxy acetyl chloride

To a 50 mL RBF charged with 4-chlorophenoxyacetic acid (0.5 g, 0.0029 mole) and THF(10 mL), was added thionyl chloride (0.34 g, 0.0043 mole) under cooling. The reaction mixture was heated at 65 ^{o}C until completion (monitored by TLC). The reaction mixture was concentrated under *vacuo* to obtain the crude product (0.56 g), which was as such taken for next step.

Aryloxy benzocaine derivative-2: N, 4-bis (3-(3-chlorophenoxy)-2-oxo-4-phenylazetidin-1-yl) benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.5 g, 0.0015 mole), triethylamine (0.7 g, 0.0076 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^{o}C, was added 4-chloro phenoxy acetyl chloride (0.56 g, 0.003 mole) in toluene (3 mL) under nitrogen atmosphere. To reaction mixture was heated for 3 hours, the reaction mixture was concentrated. The obtained residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.02 g). **LC MS:** 663.3 (M-H).

Intermediate 7: Synthesis of 4-methoxy phenoxy acetylchloride

To a 50 mL RBF charged with 4-methoxyphenoxyacetic acid (1.0 g, 0.005 moles) and THF (10 mL), was added thionyl chloride (2.37 g, 0.02 moles) under cooling. The reaction mixture was heated at 65 ^{o}C and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g), which was as such taken for next step.

Aryloxy benzocaine derivative -3: N,4-bis(3-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-1-yl)benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.4 g, 0.0012 mole), triethylamine (0.48 g, 0.0048 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^{o}C, was added and 4-methoxy phenoxy acetyl chloride (0.5 g, 0.0024

mole) in toluene (10 mL) under nitrogen atmosphere. To reaction mixture was heated for 3 hours, the reaction mixture was concentrated. The obtained Aryl residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.015 g). **LC MS:** 655.3 (M-2H).

Intermediate 8: Synthesis of 3- Bromo phenoxy acetyl chloride

To a 50 mL RBF charged with 3-Bromophenoxy acetic acid (0.5 g, 0.002 mole) and THF(10 mL), was added thionyl chloride (0.4 g, 0.003 mole) under cooling. The reaction mixture was heated at 65 ^oC and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g), which was as such taken for next step.

Aryloxy benzocaine derivative-4: N, 4-bis (3-(3-bromophenoxy)-2-oxo-4-phenylazetidin-1-yl) benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.33 g, 0.001 mole), triethylamine (0.4 g, 0.004 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^oC, was added 4-bromo phenoxy acetyl chloride (0.5 g, 0.002 moles) in toluene (10 mL) under nitrogen atmosphere. To reaction mixture was heated for 4 hours, the reaction mixture was concentrated. The obtained residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.04 g), **LC MS:** 754.0(M+H).

Intermediate 8: Synthesis of 4- Bromo phenoxy acetyl chloride-To a 50 mL RBF charged with 4-Bromophenoxy acetic acid (0.5 g, 0.002 mole) and THF(10 mL), was added thionyl chloride (0.4 g, 0.003 mole) under cooling. The reaction mixture was heated at 65 ^oC and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g), which was as such taken for next step.

Aryloxy benzocaine derivative-5: N, 4-bis (4-(4-bromophenoxy)-2-oxo-4-phenylazetidin-1-yl) benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.33 g, 0.001 mole), triethylamine (0.4 g, 0.004 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^oC, was added 4-bromo phenoxy acetyl chloride (0.5 g, 0.002 moles) in toluene (10 mL) under nitrogen atmosphere. To reaction mixture was heated for 4 hours, the reaction mixture was concentrated. The obtained residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.04 g), **LC MS:** 754.0(M+H).

Intermediate 7: Synthesis of 4-Nitro phenoxy acetylchloride

To a 50 mL RBF charged with 4-Nitrophenoxyacetic acid (1.0 g, 0.005 moles) and THF (10 mL), was added thionyl chloride (2.37 g, 0.02 moles) under cooling. The reaction mixture was heated at 65 ^oC and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g), which was as such taken for next step.

Aryloxy benzocaine derivative -5: N,4-bis(3-(4-Nitrophenyl)-2-oxo-4-phenylazetidin-1-yl)benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.4 g, 0.0012 mole), triethylamine (0.48 g, 0.0048 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^oC, was added and 4-Nitro phenoxy acetyl chloride (0.5 g, 0.0024 mole) in toluene (10 mL) under nitrogen atmosphere. To reaction mixture was heated for 3 hours, the reaction mixture was concentrated. The obtained Aryl residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.015 g). **LC MS:** 655.3 (M-2H).

Intermediate 7: Synthesis of 4-Nitro phenoxy acetylchloride

To a 50 mL RBF charged with 4-Methylphenoxyacetic acid (1.0 g, 0.005 moles) and THF (10 mL), was added thionyl chloride (2.37 g, 0.02 moles) under cooling. The reaction mixture was heated at 65 ^oC and continued until completion (monitored by TLC). The reaction mixture was concentrated to obtain the crude product (0.5 g), which was as such taken for next step.

Aryloxy benzocaine derivative -6: N,4-bis(3-(4-methylphenyl)-2-oxo-4-phenylazetidin-1-yl)benzamide.

To a 50 mL RBF charged with (4E, 10E)-N'-benzylidene-4-(benzylideneamino) benzohydrazide (0.4 g, 0.0012 mole), triethylamine (0.48 g, 0.0048 mole) in toluene (10 mL). The reaction mixture was heated at 80 ^oC, was added and 4-methyl phenoxy acetyl chloride (0.5 g, 0.0024 mole) in toluene (10 mL) under nitrogen atmosphere. To reaction mixture was heated for 3 hours, the reaction mixture was concentrated. The obtained Aryl residue was neutralized with 10% Na_2CO_3 solution, extracted with ethyl acetate. The combined extract was washed with water drier over anhydrous Na_2SO_4, concentrated to obtain crude product, which was purified by column chromatography to obtain the title product (0.015 g). **LC MS:** 655.3 (M-2H).

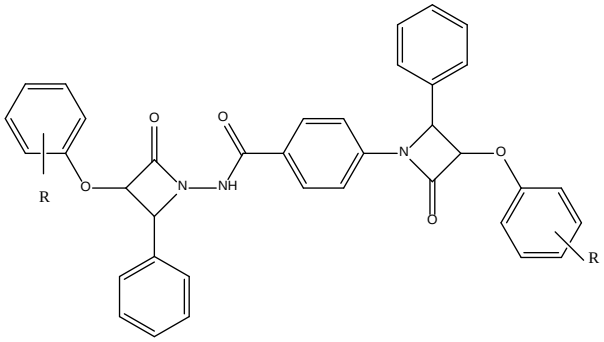
Anticancer Study

The anticancer activity of the synthesised compounds was carried out on cancer cell lines namely HT-29 (Colon cancer) and experimental work were done in Nathaji Rao G. Halgekar Institute of Dental Sciences and centre, Belgaum, Karnataka, India.The inhibition of the growth of cell lines, i.e., Cytotoxicity was considered as anticancer activity.Toxicity of test compound in cells was de termined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly coloured blue formazan product which was measured as absorbance at 492nm on a spectrophotometer (max, Molecular devices)and the IC50 value were determined by plotting % inhibition (from control) versus concentration.

Table-1

Compound	Concentration(µg/ml)	OD	AT(%) of cell Lysis	IC50
A1	10 20 30	0.532 0.566 0.692	No Lyses No Lyses No Lyses	20
A3	10 20 30	0.809 1.086 1.111	25 50 No lyses	>30
A4	10 20 30	0.532 0.566 0.692	No lyses No lyses No lyses	>30
A5	10 20 30	0.670 0.666 1.111	No lyses No lyses >75	> 30
A6	10 20 30	0.546 0.668 0.927	No Lyses No Lyses 50%	30

General structure:



Aryl benzocaine derivative	R	R1	Melting point
1	H	H	160
2	4-Cl	4-Cl	220
3	4-OCH_3	4-OCH_3	173
4	3-Br	3-Br	185
5	4-Nitro	4-Nitro	196
6	4-Methyl	4-Metyl	184

ANTICANCER ACTIVITY

Anticancer studies

The anticancer activity of the synthesizes compounds were carried out on cancer cell lines namely A549 (Lung cancer), Media DMEM with low glucose, and experimental work were done in Nathaji Rao G. Halgekar Institue of dental sciences and research centre, Belgaum, Karnataka, India. The inhibition of the growth of cell lines that is cytotoxicity was considered as anticancer activity. The toxicity of test compound in cells was determined by MTT assay on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product which was measured as absorbance at 570 nm on a spectrophotometer and IC_{50} values were determined by plotting % inhibition (from control) v/s concentration.

Synthesis of N, 4-bis (2-oxo-3-phenoxy-4-phenylazetidin-1-yl) benzamide compound: M. Wt. 563 g/mol IR cm^{-1} 3176 (NH), 1701 (C=O), 3030 (NH-C=O), 837 (Ar). H^1-NMR (DMSO) 1.044-1.242(m8H, CH_2-Azitadione), 2.366 (s, 1H, CH_2), 4.718 (s, 1H, NH-C=O). MS *m/z* (%) = 562 (M^+).

Synthesis of **N**, 4-bis (3-(3-chlorophenoxy)-2-oxo-4-phenylazetidin-1-yl) benzamide compound: M. Wt. 632 g/mol. IR cm^{-1} 3152 (NH), 1730 (C=O), 844 (Ar), 744 (C-Cl). H^1-NMR (DMSO/ppm) δ_H 1.124-1.242 (m8H, CH_2-Azitadione), δ_H 2.506 (δ 1H, Ar-CH_2), δ_H 3.175 (δ 1H, Ar-CH=NH), δ_H 4.529 (δ 1H, NH-CH=O). MS *m/z* (%) = 631 (M^+).

Synthesis of N, 4-bis (3-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-1-yl) benzamide compound: M. Wt. 623 g/mol. IR cm^{-1} 3176 (NH), 1769 (C=O), 837 (Ar), 2360 (OCH_3). H^1-NMR (DMSO) δ_H 1.079- 1.194 (m8H, CH_2-Azitadione), δ_H 2.506 (δ 1H, CH_2), δ_H 3.402 (δ, 3H, -OCH_3), δ_H 4.718 (δ 1H, NH-CH=O). MS *m/z* (%) = 621 (M^+).

Synthesis of N, 4-bis (3-(3-bromophenoxy)-2-oxo-4-phenylazetidin-1-yl) benzamide compound: M. Wt. 721 g/mol. IR cm^{-1} 3152 (NH), 1730 (C=O), 844 (Ar), 756 (C-Br). H^1-NMR (DMSO/ppm) δ_H 1.124-1.242 (m8H, CH_2-Azitadione), δ_H 2.506 (δ 1H, CH_2), δ_H 3.175(δ 1H, Ar-CH=N), δ_H 4.529 (δ 1H, NH-CH=O). MS *m/z* (%) = 720 (M^+).

Synthesis of N,4-bis(3-(4-Nitrophenyl)-2-oxo-4-phenylazetidin-1-yl)benzamide compound: M. Wt. 655 g/mol. IR cm^{-1} 1519 (NO_2), 3061 (NH), 2945 (Ar-CH), 1622 (NH-C=O). H^1-NMR (DMSO/ppm) δ_H 1.114-1.602 (m8H, CH_2-Azitadione), δ_H 2.505 (δ 1H, CH_2), δ_H 3.740 (δ 1H, Ar-CH=N), δ_H 4.601 (δ 1H, NH-CH=O), δ_H 7.129-7.926 (m8H, Ar-H). MS *m/z* (%) = 654 (M^+).

Synthesis of N,4-bis(3-(4-methylphenyl)-2-oxo-4-phenylazetidin-1-yl)benzamide compound: M. Wt. 639g/mol. IR cm^{-1} 3176 (NH), 1769 (C=O), 837 (Ar), 2360 (OCH_3). H^1-NMR (DMSO) δ_H 1.079- 1.194 (m8H, CH_2-Azitadione), δ_H 2.506 (δ 1H, CH_2), δ_H 3.402 (δ, 3H, -OCH_3), δ_H 4.718 (δ 1H, NH-CH=O). MS *m/z* (%) = 640 (M^+).

CONCLUSION :

The cycloaddition reaction of Aryloxy derivative with different Phenoxy acids to obtain Bis-azetidione derivatives (A_1-A_6) was attempted by employing various reagents and reaction conditions. However the desired cycloaddition was successful only when the reaction was carried out by using conc.H_2SO_4 as a catalyst and ethanol as a solvent. The desired Bis-azetidione derivatives (A_1-A_6) were obtained in a good yield by conventional method. The anticancer activity, of the synthesized Bis-azetidione derivatives revealed that the compounds A_3, A_4, A_5, A_2 and A_1 were effective HT-29 cell lines respectively.

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