Quest Journals
Journal of Research in Pharmaceutical Science
Volume 8 ~ Issue 8 (2022) pp: 01-06
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Synthesis of an Anaalogue of COTC and Their Evaluation of Anti-Cancer Activity

Juliana Chineze Obi
Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.
Correspondence: Juliana Chineze Obi, Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University, P.O. BOX 02, Anambra State, Nigeria.

Abstract
*Two analogues of COTC (**2a and 2aii**) were synthesized. This reaction involves an eight-step reaction sequence using a natural product (-)-quinic acid (**1a**) as a precursor. The products were obtained in moderate yields (29-67%). The purity and the identities of the compounds were confirmed using NMR, IR and mass spectrometry. The MTT assay' was carried out in A549 cancer cell line which has a high level of NQO1 activity. Remarkably, the IC_{50} results obtained revealed that an analogue of COTC (**2a**) with (IC_{50} 3.7 ± 0.2 µM) is more effective as anticancer agent when compared to compound (**2aii, IC_{50}** 21.3 ± 3.6 µM).*
Key words: *synthesis, COTC, MTT assay and spectral analysis.*

***R**eceived 19 July, 2022; **R**evised 31 July, 2022; **A**ccepted 02 August, 2022 © The author(s) 2022.*
P**ublished with open access at **www.questjournals.org

I. Introduction

A number of *anti*-cancer agents have been obtained from *Streptomyces*, the largest genus of actinobacteria (Gram-positive bacteria) which grow in different locations. A notable characteristics feature of *Streptomyces* bacteria has emerged to be their ability to produce a range of bioactive secondary metabolites which include *anti*-cancer agents, immunosuppressive agents and antibiotics (Zhang, X., *et al.*, 2012). This has increased the interest in the search for more novel bioactive secondary metabolites from the *Streptomyces* genus. One outcome of this research interest has been the discovery of the *anti*-cancer compounds: 2-crotonyloxymethyl-(4R,5R,6R)-4,5,5-trihyroxyclohex-2-enone (COTC, **3a)**, gabosine (**3b**), antheminone A (**3c**) phorbasin B (**3d**) and COMC (**3e**) as shown in Figure 1.

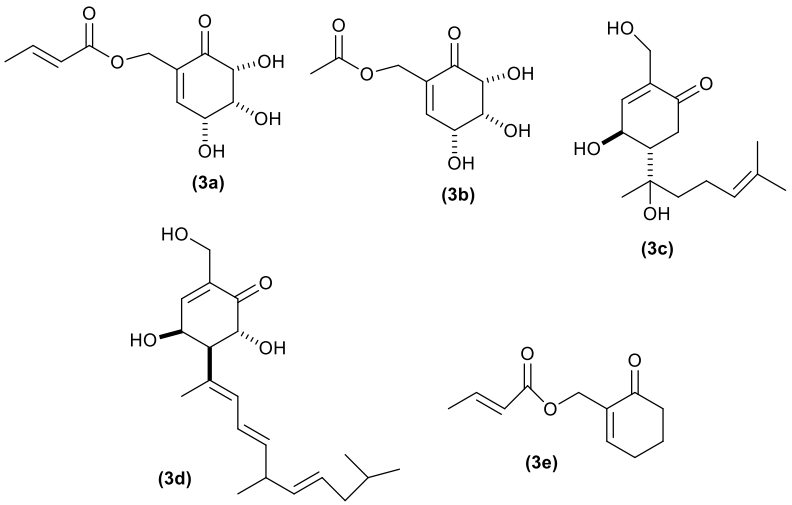


Figure 1: Structures of COTC (**3a**), gabosine (**3b**), antheminone A (**3c**), phorbasin B (**3d**) and COMC (**3e**).

COTC was found to exhibit remarkable toxicity towards some cancer cell lines in culture as well as in tumor bearing mice (Takeuchi, T., *et al.,* 1975). Further research conducted into the *anti*-cancer activity of COTC, revealed that the glyoxalase 1 enzyme obtained from the rat liver and yeast was inhibited by COTC. Glyoxalase enzyme was reported to support cell growth and regulation by controlling the level of toxic methylglyoxal (**4a**) produced by normal body metabolism (Szent-Gyorgi, A., 1968). This inhibition will result in a carcinostatic effect by preventing the metabolism of methylglyoxal in tumor cells and hence leading to cell apotosis (French, F. A. and Freedlander, L., 1958). Further research by Takeuchi and his co-workers also proved that daily injections of COTC into mice that had been intraperitoneally inoculated with cancer cells led to strong inhibition of growth of Hela cells and EHRLICH ascites carcinoma and a weak inhibition of L-1210 leukaemia cells, with low toxicity (Takeuchi, T., *et al.,* 1975). The fact that many biological agents that either initiate or retard cancer growth react with –SH containing molecules such as thiophenol (**4b**), 2-mercaptoethanol (**4c**) and cysteine (**4d**) (Figure 2), indicates that there may be some form of relationship between cancer and GSH (Takeuchi, T., *et al.,* 1975).

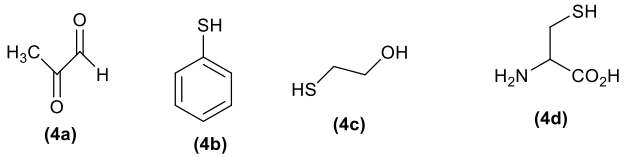
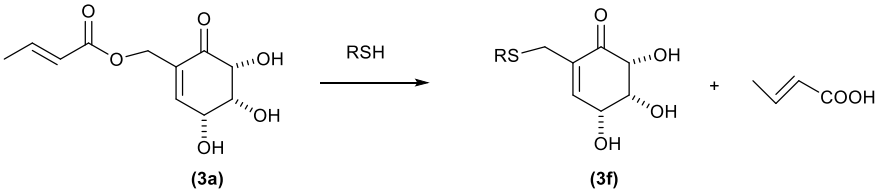


Figure 2: structures of compounds containing –SH moiety and methylglyoxal (**4a**).

Thus, the *anti*-tumor properties of both COTC (**3a**) and COMC (**3e**) indeed, were actually based on this property. In view of this, (Takeuchi, T., *et al.,* 1975), conducted extensive research into the bioactivity of COTC by studying its reaction with a series of sulfhydryl containing compounds. The team observed a rapid replacement of the crotonate group in COTC by 2-mercaptoethanol (**3f**) as depicted in Scheme 1.



Scheme 1: Nucleophilic displacement of the crotonyl moiety of COTC (**3a**) by sulfhydryl containing compounds.

The team therefore, concluded that the anti-cancer activity of COTC was as a result of the nucleophilic substitution of the crotonate group by an intracellular sulfhydryl (-SH) group as that present in glutathione (GSH). The potent anti-cancer activity of COTC prompted efforts from many researchers to carry out further investigations into compounds of that nature (**5a-d**) as demonstrated in Figure 3.

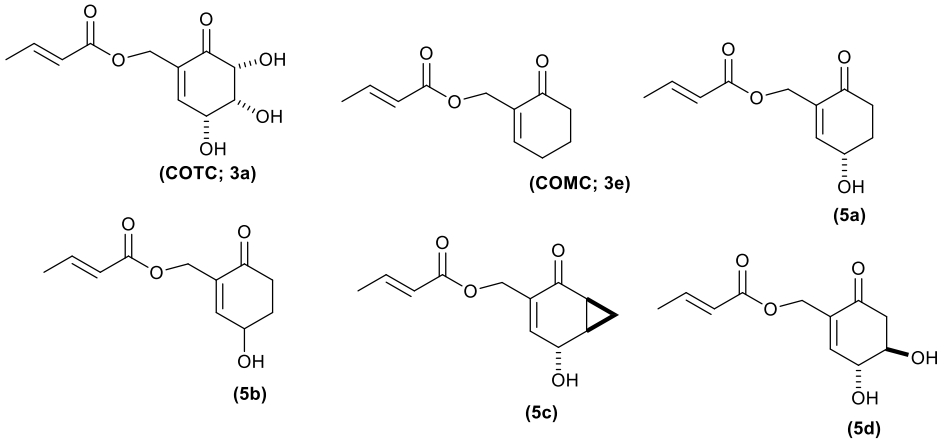


Figure 3: Structures of hydroxylated analogues of COTC **(3a).**

These analogues of natural product of COTC (**5a-d**), were synthesized in order to improve potency and /or the cytotoxicity profile. The studies conducted by (Douglas, K. T., *et al.,* 1992) revealed that the non-hydroxylated synthetic compound COMC (**3e**), was more toxic towards cancer cell lines than the trihydroxylated natural products (COTC) as shown in Table 1 (Arthurs, C. L., *et al.,* 2010).

	IC_{50} (μM)	
Compound	A549	H460
COMC (**3e**)	54.5	40.4
(**5a**)	16.7	10.9 (**5b**)
18.1	20.4	
(**5c**)	23.6	10.5
(**5d**)	147.4	158.0

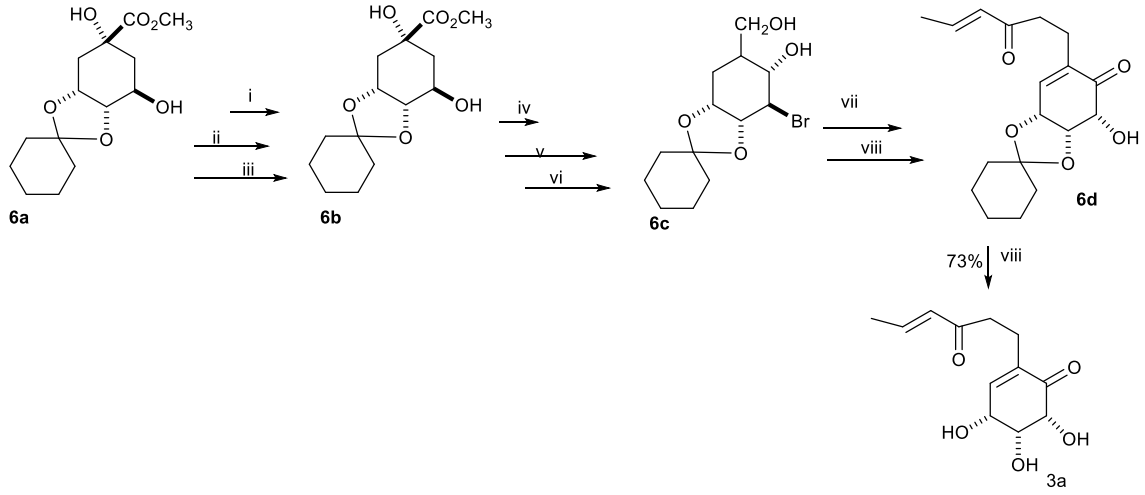
Table 1: Structures of mono-hydroxylated analogues of COTC (**5a-d**) and COMC (**3e**).

The mono-hydroxylated analogues of COTC (**5a-d**) showed remarkable toxicity towards cancer cell lines in comparison to COMC (**3e**). For example, compounds (**5a**) and (**5b**) which differ in their absolute configuration at C-4 has an insignificant effect on the potency of the compounds. But introduction of second hydroxyl group at C-5 as in compound (**5d**) decreases the efficacy of the compound towards the cancer cell lines.

This research therefore, aims at synthesizing compound that could effectively contend with COTC as an effective *anti*-cancer agent.

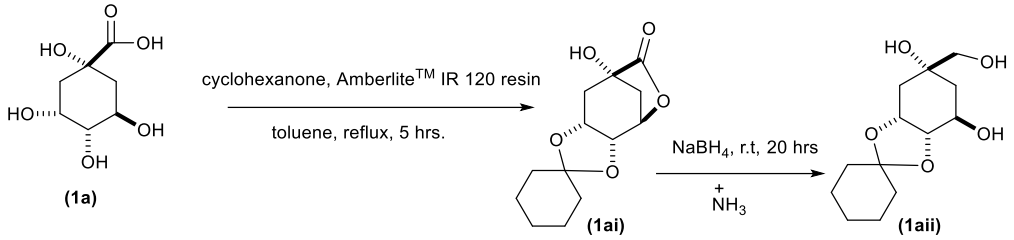
II. Results and Discussion

In 2000, Ganem and team (Frederick, C., *et al.,* 2000) reported an efficient method for the synthesis of COTC, which involved an eight-step reaction sequence which gave overall yield of 73% as depicted in Scheme 2.



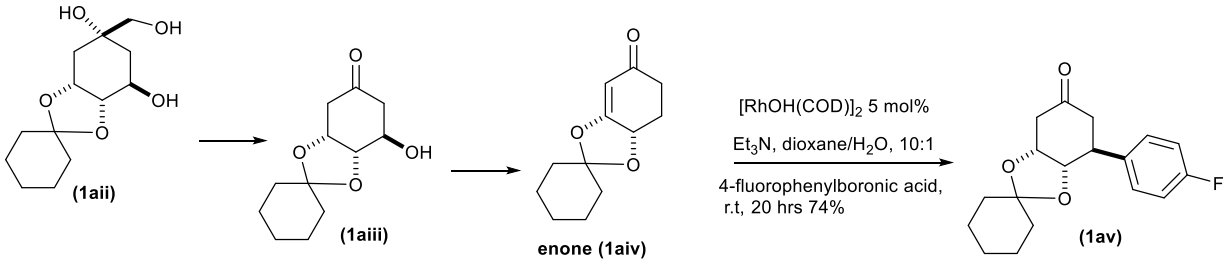
Scheme 2: Reagents and conditions; (i) Tf_2, py, DCM, 65%; (ii) CsOAc, DMF; (iii) NBS-H_2O, DMF, 72% over 2 steps; (iv) DIBAL-H, benzene-toluene, 65%; (v) $LiN(TMS)_2$, THF, -78 ^oC, 87%; (vi) CH_3SO_3H, DMSO, R.T., 1.5 hr then Et_3N, r.t., 5 mins, 71%; (vii) crotonic anhydride, DCC, DMAP, THF, 54%; (viii) 1:1 TFA:H2O, 73%.

The conversion of (-)-quinic acid (**1a**) to cyclohexylidene quinide followed a modiefied sequence by boiling a mixture of (-)-quinic acid, cyclohexanone and acid catalyst (Amberlite™ IR 120 (H) resin) in a mixture of benzene and DMF (Mercier, D., *et al.,* 1971). An alternative procedure was utilized by using toluene as a reaction medium due to the health hazard linked with the use of benzene. In this reaction, the cis-1,2-diol group of (-)-quinic acid (1a) was selectively protected with cyclohexanone in the presence of acid catalyst (Amberlite™ IR 120 (H) resin) to give cyclohexylidene ketal (**1ai**) as shown in Scheme 3.



Scheme 3: Synthesis of cyclohexylidene ketal (**1ai**) and its reduction using $NaBH_4$ in methanol to give triol (**1aii**).

The reductive ring opening of Υ-lactone (**1ai**) was achieved by adopting the procedure described by Schulz and his team (Schulz*, et al.,* 2000). Before then, reduction of ester and lactones is carried out using a strong reducing agent such as lithium aluminum hydride, but the group modified this by using mild reducing agent $NaBH_4$ in methanol solvent to give triol (1**aii**). The reduction was successful due to the presence of electron withdrawing hydroxyl group adjacent to the carbonyl which increases its electrophilic nature and reactivity towards nucleophilic attack by the hydride ion (Obi, J. C., 2022). The oxidative cleavage of compound (**1aii**) was accomplished using sodium periodate ($NaIO_4$). Although this reagent has its limitations, such as poor solubility in apolar solvents. But this problem was solved by using free flowing powder form of silica gel-supported $NaIO_4$ (Arthurs, C. L., 2010) to give hydroxyl ketone (**1aiii**) as shown in Scheme 4. Application of Danishefsky and co-workers method (Audia, J. E., 1989), was used for the elimination of β-hydroxyl using methanesulfonyl chloride in the presence of trethylamine to give enone (**1aiv**). 1,4-conjugate addition was carried using a procedure adopted by Miyuara and co-workers (Sakai, M. *et al.,* 1997). The group reported the first catalytic conjugate addition of aryl and alkenyl boronic acids reaction to an enone catalyzed by a rhodium (1) complex. The rhodium(1) complex was generated in situ *via* the reaction of $[Rh(acac)(CO)_2]$ and an achiral phosphine ligand. This reaction was carried out in the presence of Et_3N and dioxane/H_2O (10:1) to give fluorophenyl adduct (**1av**) in good yield as shown in Scheme 4.



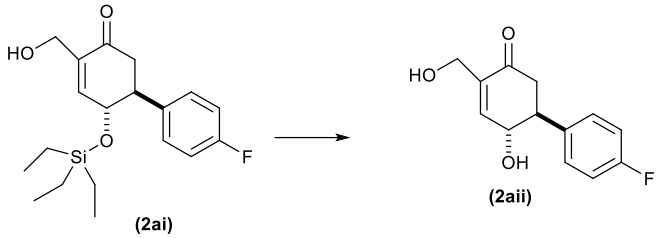
Scheme 4: Synthesis of fluorophenyl adduct (**1av**).

The final step involves the application of Morita-Baylis-Hillman (MBH) reaction which was first reported in nineteen seventies, is one of the popular reactions for the formation of a C-C bond using an organocataylst. The MBH reaction combines both adol and Michael reactions in a single step to yield compounds with multiple functionalities. For example, the products have been used as precursors in the synthesis of medicinally important pharmaceutical compounds (Das, B., *et al.*,2006; Amarante, W., *et al.,* 2008) as well as natural products (Wasnaire, P., 2006). In this research work described herein however, MBH adduct was used for the synthesis of *anti*-cancer agent (**2a**).

III. Experimental

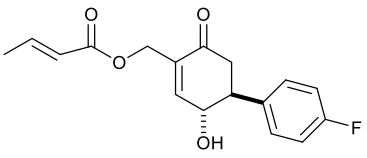
All the reagents used were obtained from commercial sources (Sigma-Aldrich Co., Alfa Aesar and Fisher Scientic). Melting point was measured using a Sanyo Gallenkamp MPD. 350 variable heater instrument and are uncorrected. IR spectra were recorded in solid state using a Bruker Alpha PFT-IR instrument. ^{13}C and ^{1}H NMR were recorded using Bruker Avance 400 spectrometers. Chemical shifts are given in ppm to the nearest 0.01 ppm and referenced to the solvent residual peak. Proton assignments were assisted by DEPT. HCOSY and ^{1}HMQC.

Synthesis of (1S,6R)-4'-fluoro-6-hydroxy-4-(hydroxymethyl)-1,6-dihydro-[1,1'-biphenyl]-3(2H)-one (2aii)



To a suspension of compound ((**2ai**) (49 mg, 0.14 mmol) in TFA: H_2O (7:1. 1 mL) was stirred for 30 mins at room temperature. The solvent were concentrated in *vacuo* to give a crude brown oil which was purified by flash silica chromatography (ethyl acetate : petroleum (2:1) to give compound (**2aii**) as a colorless film (22 mg, 67%): V_{max} (film/cm^{-1} 3347, 1668; δ_H (400 MHz; $CDCl_3$) 2.65-2.76 (2H), 3.24 (1H), 4.30-4.40 (2H), 4.67 (1H), 6.95 (1H), 7.06-7.12 (2H), 7.26 – 7.29 (2H); δ_C (100 MHz; $CDCl_3$) 43.4, 49.9, 61.0, 71.9, 116.0, 129.2, 135.0, 147.9, (C4')F) is not visible, 198.4; δ_F (376 MHz; $CDCl_3$) -114.2 (s); *m/z* (+ES) 259 ([M + Na]$^+$, 100%; Found 259.0759, $C_{13}H_{13}Na]^+$), requires 259.0746).

Synthesis of (1S,6R)-4'-fluoro-6-hydroxy-3-oxo-1,2,3,6-tetrahydro-[1,1'-biphenyl]-4-yl)methyl (*E*)-but-2-enoate (2a).



To a solution of alcohol (2**aii**) (88 mg, 0.25 mmol) in dichloromethane (1 mL), was added DMAP (3.5 mg, 0.03 mmol), pyridine (0.2 mL, 2.26 mmol) and crontonic anhydride (82 µL, 0.56 mmol). The reaction mixture was left to react at room temperature with stirring for 2.5 hours. The reaction was quenched by the addition of a saturated aqueous solution of $NaHCO_3$ (2 mL) and diluted with water (3 mL) and dichloromethane (5 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$ and evaporated in *vacuo* to give crude brown oil. This crude was purified by flash silica chromatography (ethyl acetate: petroleum ether 1:9); *Rf* 0.32 to give a colourless oil (35 mg). A solution of crude oil (35, 0.09 mml) in TFA:H_2O (7:1. 2 mL) was stirred at room temperature for 45 minutes. The solvents were evaporated in *vacuo* to give the crude product as brown oil. This crude product was purified by flash silica chromatography (ethyl acetate : petroleum ether 1 : 2): *Rf* 0.4 to give the title compound (**2a**) as a colourless thick oil (29 mg, 38% over 2 steps): $V_{max/}$ (film cm^{-1} 3432, 1714, 1675; δ_H (400 MHz; $CDCl_3$) 1.91 (3H), 2.66-2.76 (2H), 3.25 (1H), 4.67 (1H), 4.85N(1H), 4.91 (1H), 5.90 (1H), 6.94 (1H), 7.00-7.04 (2H), 7.08-7.12 (2H), 7.26-7.29 (2H); δ_C (100 MHz; $CDCl_3$) 18.1, 43.3, 49.8, 60.1, 71.9, 116.1, 122.1, 129.2, 134.3, 145.8, 148.0, (C(4)F) is not visible, 165.9, 196.1; δ_F (376 MHz; $CDCl_3$) -114.1; m/z (+ES) 327.1 ([M+Na]$^+$, 100%; Found 327.1016, $C_{17}NaO_4F$ ([M +Na]$^+$), requires 327.1009.

IV. **MTT cell viability Assay**

An IC_{50} value, in terms of an enzyme assay, represents the concentration of a drug that is required for 50% inhibition in vitro, whereas, in terms of cytotoxicity, it represents the concentration of a drug required to inhibit the growth of cells by 50%. For the MTT cell viability assay, it represents the concentration at which half of the cells seeded remain viable at the end of the analysis. The MTT cell viability assay was carried out on compound (**2a**) an analogue of COTC and antheminone A (**2aii**) as shown in Figure 4.

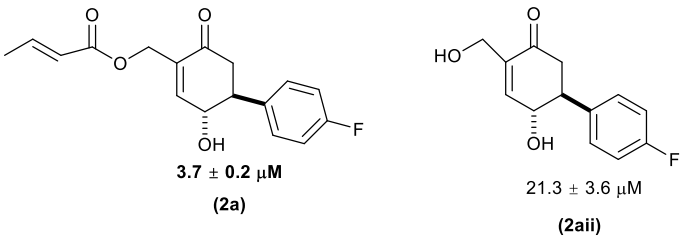


Figure 4: IC_{50} values for the synthetic analogues of COTC (2a) and anthemionone A **(2aii).**

The COTC analogue (**2a**) display higher toxicity towards the A549 cancer cell line than the antheminone A analogue (**2aii**) earlier reported by (Obi, J. C., 2022). The reason for this remarkable difference in the IC_{50} values may be due to the poor leaving group ability of the OH group in (**2aii**) which consequently cannot be displaced by GSH.

V. Conclusion

The use of cytotoxic drugs is an obvious therapeutic means for the treatment of cancer. The result of the cytotoxicity bioassay of the compounds (**2a** and **2aii**) against non-small cancer cell lines A549 could provides useful information, which will help in the guide for the future design of the compounds in that class with enhanced *anti*-cancer activity.

Acknowledgement

I am grateful to the TETfund Nigeria, for sponsoring this research abroad. I am also thankful to the staff of Chemistry department, University of Manchester, England for providing all the necessary materials and equipments during this research work.

References

[1]. Zhang, X., Chen, L. X., Ouyang, L., Cheng, Y. and Liu, B. *Cell Prolif*., 2012, **45**, 466-476.
[2]. Takeuchi, T., Chimura, H., Hamada, M., Umezawa, H., Yoshioka, O., Oguchi, N., Takahshi, Y. and Matsuda, A .J., *Antibio*., 1975, **28**, 737-742.
[3]. Szent-Gyorgi, A., *Science*, 1968, **161**, 988-990.
[4]. French, F. A. and Freedlander, B. L., *Cancer Res*., 1958, **18**, 172-175.
[5]. Douglas, K. T., Philips, R. M. and Shing, K. M., *Anti-cancer Drug Des*., 1992, **7**, 67-75.
[6]. Arthurs, C. L., Morris, G. A., Piacenti, M., Pritchard, G., Strtford, I. J., Tatic, T., Whitehead, R. C., Williams, K. F. and Wind, N. S., *Tetrahedron*, 2010, **66**, 9049-9060.
[7]. Collu, F., Bonsignore, L., Casu, M., Floris, J., Gertsch, J. and Cottiglia, F. Giorg. *Med. Chem. Lett*., 2008, **18**, 1559-1562.
[8]. Frederick, C., Huntley, M., Wood, H. B. and Ganem, B., *Tetrahedron Lett*., 2000, **41**, 2031-2034.
[9]. Mercier, D., Leboul, J., Ceophax, J. and Gero, S. D., *Carbohydr. Res*., 1971, **20**, 299-304.
[10]. Schulz, J., Beaton, M. W. and Gani, G., *J. Chem. Soc*., *Perkin Trans*., 2000, **1**, 943-945.
[11]. Obi, J. C., *international Journal of* Chemistry, 2022, **14**, 1-7.
[12]. Audia, J. E., Biosvert, L., Patten, A. D., Villalobos, A. and Danishefsky, S. J., *J. Org.Chem*.,1989, **54**, 3738-3740.
[13]. Sakai, M., Hayashi, H. and Miyaura, N., *Organometallics*, 1997, **16**, 4229-4231.
[14]. Das, B., Chowdhury, N., Banerjee, J., and Anjoy, M., *Tetrahedron Lett*., 2006, **47**, 6615-6618.
[15]. Amarante, G. W., Rezende, P., Cavallaro, M. and Coelho, F., *Tetrahedron Lett*., 2008, **49**, 3744-3748.
[16]. Wasnaire, P., Wiaux, M., Touillaux, R. and Marko, I. E., *Tetrahedron Lett*., 2006, **47**, 985-989.