

# Aryl sulfonate based anticancer alkylating agents

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**Abstract:** This research work revolves around synthesis of antineoplastic alkylating sulfonate esters with dual alkylating sites for crosslinking of the DNA strands. These molecules were evaluated as potential antineoplastic cross linking alkylating agents by reaction with the nucleoside of Guanine DNA nucleobase at both ends of the synthesized molecule. Synthesis of the alkylating molecules and the crosslinking with the guanosine nucleoside was monitored by MALDI-TOF mass spectroscopy. The synthesized molecule's crosslinking or adduct forming rate with the nucleoside was compared with that of 1,4 butane disulfonate (busulfan), in form of time taken for the appearance of  $[M+H]^+$ . It was found that aryl sulfonate leaving group was causing higher rate of nucleophilic attack by the Lewis basic site of the nucleobase. Furthermore, the rate was also found to be a function of electron withdrawing or donating nature of the substituent on the aryl ring. Compound with strong electron withdrawing substituent on the para position of the ring reacted fastest. Hence, new alkylating agents were synthesized with optimized or desired reactivity.

**Keywords:** Organic synthesis, alkylation, MALDI-MS, antineoplastic, busulphan.

## INTRODUCTION

Alkylating agents belong to one of the earliest developed chemotherapy drugs. They work by reacting with nucleophilic sites of the DNA nucleobases, bonding alkyl groups to them. This prevents the uncoiling and replication DNA strands, as a result the cancer cell dies. General Classes of antineoplastic alkylating agents include Nitrogen Mustards, Nitrosoureas, Azirdines, Alkyl sulfonates, Platinum Coordinate complexes (Sriram and Yogeewari, 2009). Among the sulfonate based alkylating agents, 1, 4-butanediol dimethanesulfonate (Busulfan) is the most common molecular structure, consisting of esters of methanesulfonic acid. Some work has been reported on structural derivatives of busulfan (Menuel *et al.*, 2007) and other sulfur derivatives such as sulfides, sulfones (Santelli-Rouvier *et al.*, 2004). Antineoplastic alkylating agents have Lewis acidic sites which get attacked by Lewis basic sites of DNA bases which get alkylated. The common Lewis basic site of DNA is the  $sp^2$  hybridized N-7 of purine ring of Guanine base. Usually, alkyl group gets bonded to the guanine base of DNA, at the number N-7 atom of the purine. Other potential nucleophilic or Lewis basic sites in DNA bases are N-1 and N-3 of adenine, N-3 of cytosine, O-6 of Guanine. Sulfonyl chloride, the activated form of sulfonic acid with more stable leaving group, was reacted with 1, 4 butanediol to form aryl sulfonate ester derivatives (3a-c) (Scheme 1). There are numerous examples in literature for sulfonates ester synthesis (Al Majid 2010; Kim and Jang, 2008; Lei *et al.*, 2015). Guanosine nucleoside had number of Lewis basic sites which needed to be protected to make sure that only N-7 reacts (Scheme 1). Rate of the adduct formation was comparatively evaluated for (3a-c)

against busulfan by continuous monitoring of the adduct formation reaction. Reaction rate was measured in form of time taken for the appearance of the adduct formation pseudomolecular ion peak MALDI-TOF spectra.

## MATERIALS AND METHODS

### Materials

Reagents and solvents were obtained from Merck Millipore and Sigma-Aldrich Chemical Company, USA. All the solvents were of analytical grade and were dried using standard methods. MALDI-TOF was recorded on Ultraflex III mass spectrometer. All reactions and purity were monitored by thin layer chromatography (TLC), performed on Merck pre-coated silica gel 60 F<sub>254</sub> 20 x 20 cm aluminum sheets and spots seen under UV light at 254 and 366 nm.

### Synthesis of sulfonate ester (3a-c)

Procedure described below was same for all aryl sulfonyl chlorides and diol combinations. Here reaction detail of 1, 4-butanediol with benzenesulfonyl chloride to form (3a) is given as example. (Scheme 1). 1, 4-butanediol (2) (38.16 mmol) was stirred in dichloromethane (20 ml) under inert atmosphere of N<sub>2</sub>. Benzenesulfonyl chloride (1) (76.32 mmol) along with triethylamine (76.32 mmol) base was added to 1, 4-butanediol solution. The reaction mixture was stirred at 70°C for 6h and then brought down to the room temperature. Reaction progress was monitored using TLC and MALDI-TOF spectroscopy after every 5 minutes. The obtained reaction mass was extracted with dichloromethane (2 × 70 ml), washed with Na<sub>2</sub>CO<sub>3</sub> (0.38 M, 200 ml) solution and H<sub>2</sub>O (100.0 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The obtained product mass was further purified by column chromatography

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(Ethyl acetate: Hexane). Other sulfonate esters (3a) were synthesized using same procedure. Synthesized molecules were structurally confirmed and analyzed by MALDI-TOF. Table 1 lists the physical and spectral data for (3a-c).

**2)-[9-[(3S,4S,5S)-3,4-bis[[tert-butyl(dimethyl)silyl]oxy]-5-[[tert-butyl(dimethyl)silyl]oxymethyl]tetrahydrofuran-2-yl]-7,8-dihydro-3H-purin-6-one (6)**

Protection of -OH sites were carried out by method described in literature (Costentin *et al.*, 2010).

**N-[9-[(3S,4S,5S)-3,4-bis[[tert-butyl(dimethyl)silyl]oxy]-5-[[tert-butyl(dimethyl)silyl]oxymethyl]tetrahydrofuran-2-yl]-6-oxo-7,8-dihydro-3H-purin-2-yl]-2,2-dimethylpropanamide (8)**

Synthesis of this molecule was carried out by method described in literature (Dey and Garner, 2001)

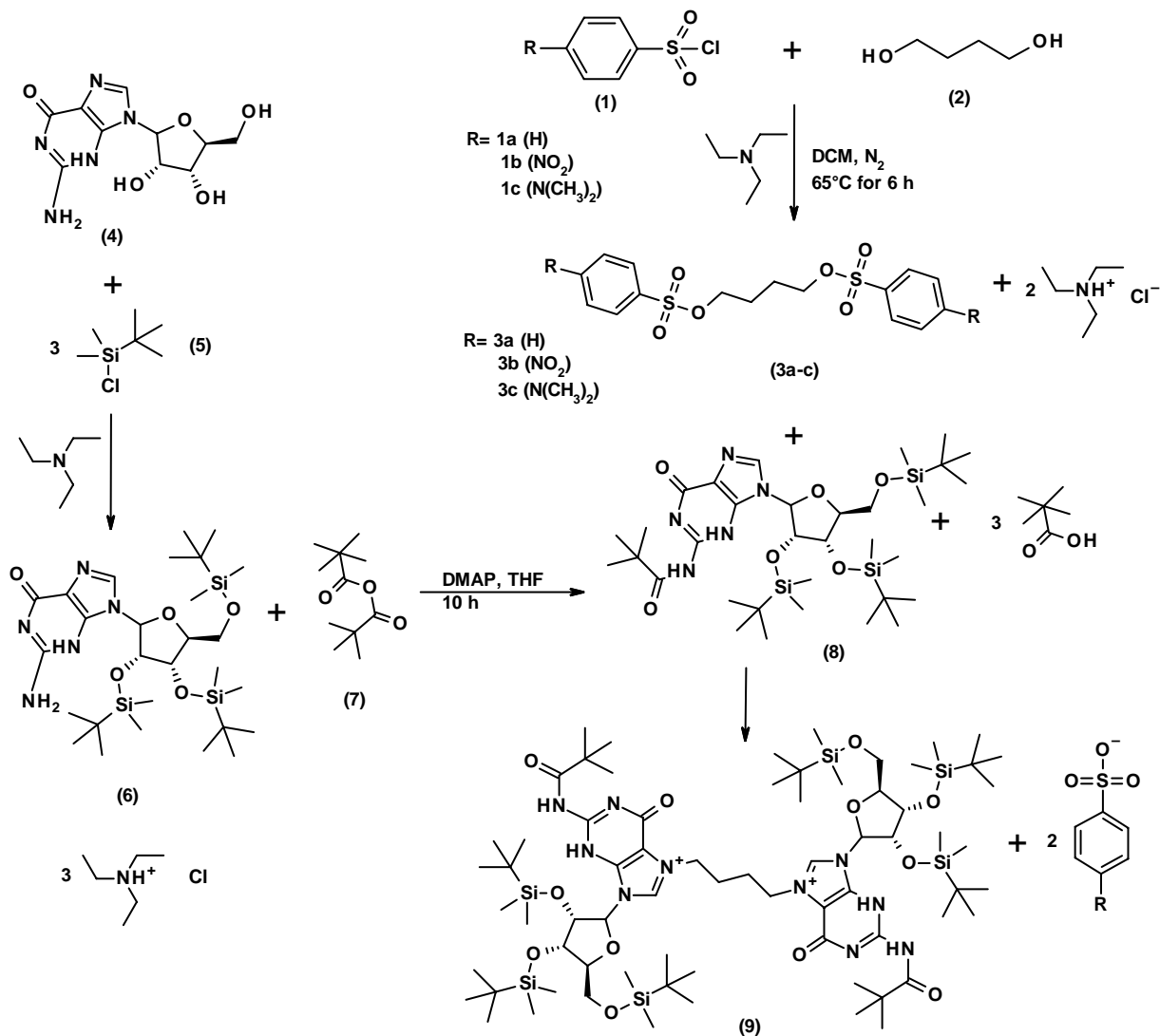
**Sulfonate ester reaction with protected nucleoside (5a-c)**

Reaction design was based upon usual conditions for SN2 mechanism in polar aprotic solvent. Adduct formation and evaluation by MALDI-MS is described in literature (Garaguso, *et al.*, 2010). Sulfonate esters (3a-c) (10 mmol) was dissolved in tetrahydrofuran with protected Guanosine (8) (10 mmol) and stirred for 18 hours at 37°C in the dark. Reaction progress was monitored using TLC. The reaction mass was analyzed by MALDI-TOF Mass spectrometry after every 5 minutes to monitor the  $[M+H]^+$ . MALDI matrix preparation protocol was used as reported in literature (Garaguso, *et al.*, 2010).

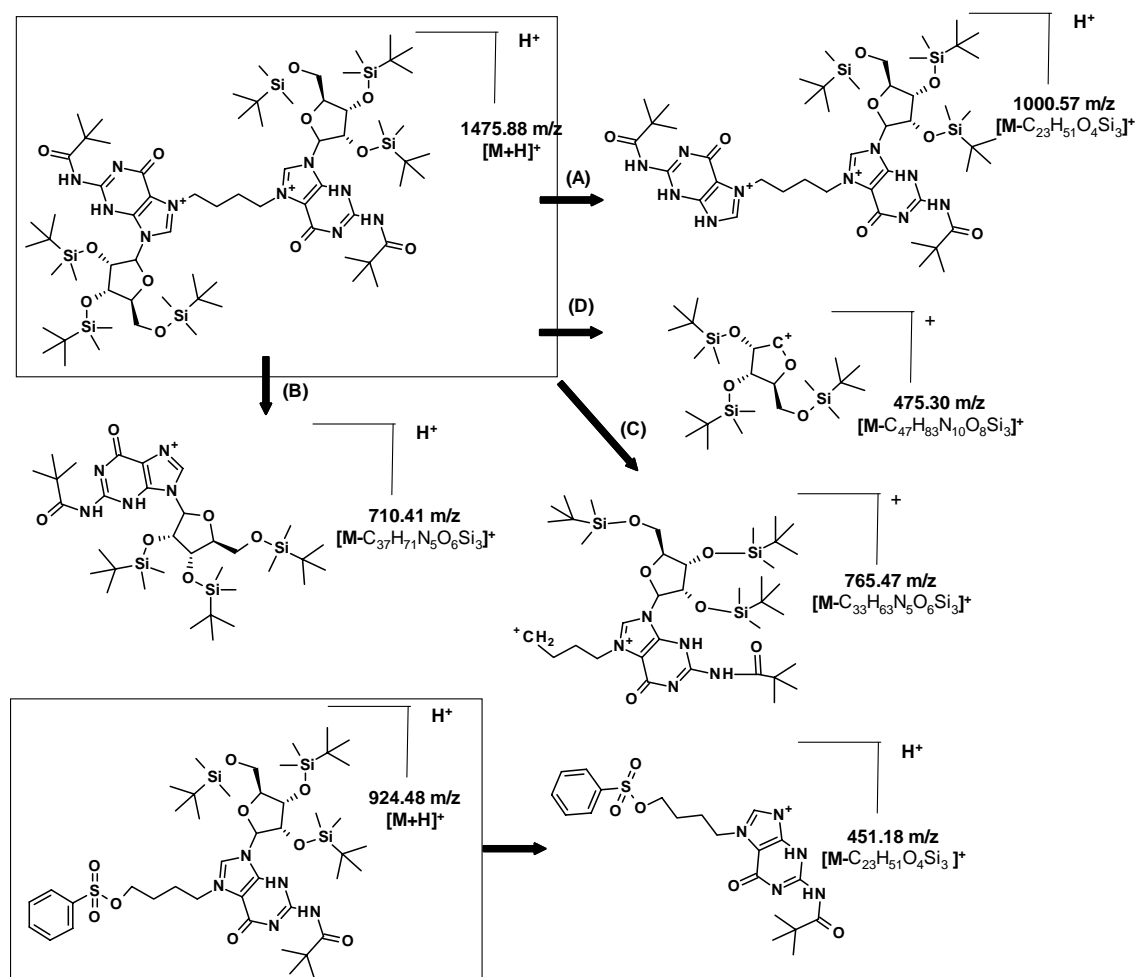
**RESULTS**

**MALDI-TOF characterization**

MALDI-TOF adduct fragmentation is shown in fig. 1. The adduct product yielded pseudomolecular ion  $[M+H]^+$  at 1475.88 m/z. Fragmentation (A) occurred by inductive



**Scheme 1:** Synthetic route for compounds (3a-c)



**Fig. 1:** MALD-MS Fragmentation pathway

cleavage of N-9 with furan ring yielding fragmentation peak 1000.57 m/z. The facile loss of furan ring is characteristic of guanine nucleoside (Garaguso *et al.*, 2010). The second most intense peak is that of loss of whole protected nucleoside itself. Inductive cleavage of N-7 with  $\alpha$  carbon of the alkylation agent yielded the molecular fragment at 710.41 m/z labeled as (B) corresponding to the loss of whole protected guanosine. Loss of furan moiety at N-9 yielded the fragment at m/z. (C) at 765.47 m/z was attributed to loss of guanosine moiety at one end of the adduct. Peak at 475.30 m/z was that of the fragment of furan ring only (D). Furthermore specific, in case of (3a) alkylating agent's pseudomolecular ions were present at 371.06 m/z with inductive fragmentation at  $\alpha$  carbon yielded the fragment at 213.05 m/z. Furthermore specific, protected nucleosides pseudomolecular ion appeared at 710.41 m/z. Monoalkylated adducts (from 3a) were also detected at 924.48 m/z. In the course of reaction, the monoalkylated adducts peak appeared earlier than bialkylated adducts, implying that more duration given to the reaction mass might have converted mono alkylation adducts to bi.

Monoalkylated adducts showed fragmentation by loss of furan moiety at 451.18 m/z. All of these fragments correspond to the Stevenson rule of forming most stable ion (Pavia *et al.*, 2001).

#### Kinetics

All of the synthesized molecules showed same pseudomolecular ions of 1475.88 m/z as Busulfan since the Lewis acidic alkyl part of the synthesized molecules (3a-c) and Busulfan is same. The only impact on rate of nucleophilic attack is that of leaving group i.e. aryl ring. Being more electron withdrawing group than alkyl group (-CH<sub>3</sub>) of busulphan, the conjugated base of aryl sulphonic acid is more stable as leaving group. That was confirmed by quicker appearance of [M+H]<sup>+</sup> adduct in the entire molecule at 10 mins (3b) as compared to the busulphan at 20mins. The -NO<sub>2</sub> group wielding aryl group (3b) is most electron withdrawing. It gave the [M+H]<sup>+</sup> peak fastest at 5 mins after commencing of the reaction. Having electron donating group at para position, the aryl ring becomes more electron rich by resonance and as result less electron withdrawing which slows the

**Table 1:** Physical and spectral data for (3a-3c)

Compound	Yield	[M+H] <sup>+</sup>	Elemental Analysis found(Calc.)				
	%	m/z	C%	H%	N%	O%	S%
4-(benzenesulfonyloxy)butyl benzenesulfonate (3a)	55	371.45	51.85 <sup>a</sup> (51.88) <sup>b</sup>	4.90 (4.90)		25.93 (25.91)	17.32 (17.31)
4-(4-nitrophenyl)sulfonyloxybutyl 4-nitrobenzenesulfonate (3b)	60	461.03	41.72 (41.74)	3.53 (3.50)	6.09 (6.08)	34.77 (34.75)	13.90 (13.93)
4-[4-(dimethylamino)phenyl] sulfonyloxybutyl 4-(dimethylamino)benzenesulfonate (3c)	82	457.14	52.64 (52.61)	6.19 (6.18)	6.19 (6.14)	21.0 (21.03)	14.01 (14.05)

a = Experimental value, b = Calculated value

**Table 2:** Rate of reaction of the adduct formation

Compound	Yield of adduct	m/z	Time of appearance of [M+H] <sup>+</sup> (Min)				
			5	10	15	20	25
5a	55	625.26	0	13	15	30	35
5b	60	625.26	4	33	45	75	77
5c	82	625.26	0	0	15	18	22
butane 1,2-diphenyl sulfonate	56	625.26	0	0	0	14	15

rate of reaction. This was confirmed by the slowest reaction and appearance of [M+H]<sup>+</sup> adduct peak after 15 mins. This proves that the rate of alkylation in sulfonate bases leaving group depends upon the electron withdrawing or donating character on the aryl ring of sulfonate.

## DISCUSSION

There are numerous recent examples of synthesis of new alkylating agents derivatives in literature (Acharya and Bansal, 2017; Chang *et al.*, 2017; Elmas *et al.*, 2017; Zuravka *et al.*, 2015). This research work encompasses structural modification of aryl sulfonate ring to reach the optimum best structure for alkylation with improved options for fine tuning the reactivity. The substituted aryl sulfonate leaving group is far more stable than an alkyl sulfonate group of busulfan because of electron withdrawing effect of aryl ring. Aryl sulfonate conjugate base stability can be further enhanced with electron withdrawing groups at ortho and para positions. Ortho substituent was not used in this research work because it can become non-coplanar because of steric hindrance with sulfonyl group. Reaction with protected nucleoside of Guanine nucleobase of DNA determined the reactivity potential of every synthesized aryl sulfonate derivative with Lewis basic site of Guanine.

This analysis scheme also evaluated the crosslinking capability of the synthesized molecules by forming adducts (5a-5c). Synthesis was accomplished by reaction of different aryl sulfonyl chlorides (1a-c) with a diol (2) molecule. While, crosslinking capability of the molecule

was evaluated by reaction with nucleoside of Guanine DNA base. Reaction with nucleoside revealed which of the synthesized molecules reacts quickest and with highest yield compared to Busulfan. There was same partial + charge on  $\alpha$  carbon atom of the alcohol part of the ester all the derivatives regardless of the electron withdrawing or donating nature substituent on the sulfonate aryl ring. Hence, change in the rate of the reaction of design molecule was only the function of the substituent on the aryl sulfonate ring or the stability of the leaving group (table 2). Other than N-7 of purine ring all other non bonding electrons on N atoms are part of 4N +2 (Huckel rule) aromaticity. Hence N-7 is the only Lewis basic N with its non bonding electrons pair in  $sp^2$  orbital. Its attack on the Lewis acids does not disrupt the aromaticity of the purine rings. Protection of Guanosine's -OH on furan ring and -NH<sub>2</sub> on purine ensures that only N-7 gets to behave as nucleophile with the alkylating agents (3a-c). Fine tuned molecular structure with enhanced reactivity was achieved by using the electron withdrawing or donating groups substituted aryl rings. Structural derivatives evaluated the effect of different substituents on aryl ring on overall reactivity of the molecule. Electron withdrawing substituent at para position is supposed to stabilize the sulfonate anion as leaving group which can increase the susceptibility of the ester molecule towards attack by Lewis basic site of DNA nucleobase.

## CONCLUSION

We designed and synthesized alkylating Lewis acid molecules for alkylation of the nucleobases of the DNA.

Rate of the alkylation were evaluated by comparison of times of appearance of MALDI TOF relative abundance of the adduct formation peaks. Rate was found to be related to the stability of the leaving group, more specifically to the substituent on the para position of the aryl ring. Compound (3b) with strong electron withdrawing substituent on the para position of the ring reacted fastest. Further structural manipulation can establish more control over the reactivity.

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