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ETOPOSIDE(VP16): CHEMICAL REACTIVITY OF ETOPOSIDE ORTHO-QUINONE WITH AMINES AND THIOLS

Dolatrai M. Vyas*, John F. Kadow, Karen L. LeBoulluec, Mark G. Saulnier,
and Terrence W. Doyle
Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492

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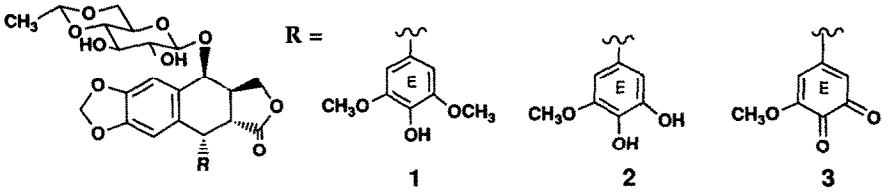
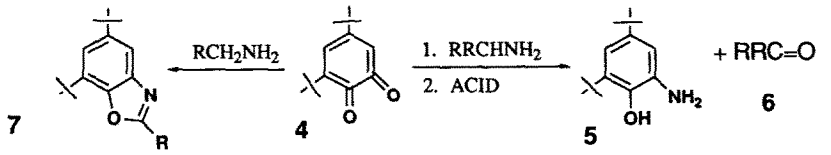
ABSTRACT: *Reaction of etoposide ortho-quinone with primary amines results in products derived from transamination and reductive amination pathways. In contrast, thiols afford Michael adducts and/or thiol-quinone redox products.*

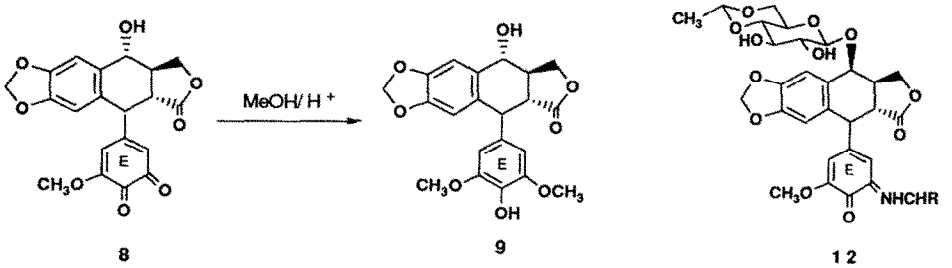
Etoposide (**1**,VP16) is an efficacious clinical antitumor agent currently employed both in combination chemotherapy and as a single agent for the treatment of several neoplasms.[1] The most widely accepted mechanism for the antitumor action of **1** involves the inhibition of topoisomerase II mediated DNA ligation.[2] However, its metabolites namely, 3'-demethyl etoposide (**2**) and the corresponding ortho-quinone **3**



have also been postulated to be involved in its mechanism of cytotoxicity.[3] In this vein, Mans and co-workers[4] have demonstrated that both **2** and **3** bind strongly to purified DNA in-vitro and can also inactivate both single and double stranded Ø 174DNA through strand cleavages stemming from the redox chemistry of these metabolites. In our preliminary evaluation in the murine P388 leukemia tumor model, **2**[5] and **3**[6] have displayed in vivo activity comparable to etoposide. To gain further insight into the chemical role of the bioactive metabolite **3**, we undertook a qualitative study to define its reactivity towards amine and thiol nucleophiles. Michael type nucleophilic reactions[7] of orthoquinones with amines and thiols have been implicated in the antitumor activity of ellipticines[8] and some substituted catechols[9], the neurotoxicity of serotonin[10], and the hypersensitivity reaction of poison ivy[11]. Interestingly, substituted ortho-quinones which do not display Michael type reactivity undergo redox chemistry instead. For example, 3,5,-di-tert butyl 1,2-benzoquinone **4** has been shown by Corey and



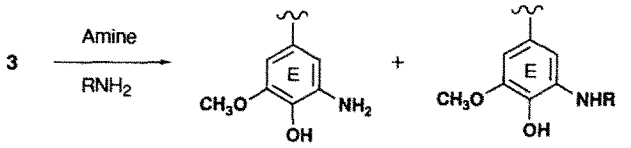
Achiwa[13] to undergo a trans-amination reaction with sec-primary amines to yield aminophenol **5** and ketone **6** upon subsequent hydrolysis. In contrast, simple primary amines yield predominantly the benzoxozoline derivative **7**. In this context, it is noteworthy that podophyllotoxin ortho-quinone **8** is also a strong oxidant, affording formaldehyde (not isolated) and methylated product **9** upon reaction with methanol under acidic conditions.[13]



8 **9** **12**

In light of the above discussion, we now report that etoposide ortho-quinone **3** displays somewhat unique reactivity towards amines. Table 1 lists the amines used, reaction conditions employed, and the products isolated. It should be stressed that in all reactions multiple products were discerned by thin layer chromatography, but only

TABLE 1. REACTION OF 3 WITH AMINES



10 **11**

Entry	Amine (R=)	Rx Conditions	Products† (% Yield)
1	Me (excess)	H_2O/1 hr	10(17):11a(20)
2	Bu (15 equiv.)	MeOH/0.5 hr	10(25):11b(18)
3	Bu (1.1 equiv.)	CH_2Cl_2/1 hr	10(27):11b(25)
4	Bu (1.1 equiv.)	MeOH/1 hr	10(25):11b(15)
5	C_6H_{11} (1.1 equiv.)	MeCN/0.5 hr	10(5):11c(12)
6‡	$PhCH_2$ (1.1 equiv.)	MeOH/0.5 hr	10(12):11d(14)

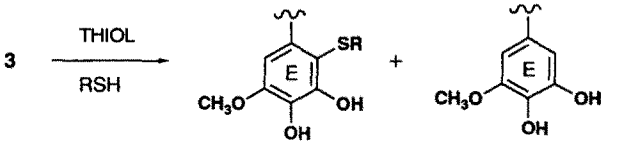
†These represent isolable products only. ‡compound 2 was also isolated in 30% yield.

isolable major products were obtained by either preperative tlc and/or column chromatography. All reactions were carried out at ambient temperature and under nitrogen atmosphere. Perusal of the table reveals that all amines yield products stemming from trans amination and reductive amination reactions. The absence of Michael addition product(s) is not surprising since the quinone substitution pattern precludes such a reaction; identical

behaviour is demonstrated by the disubstituted ortho-quinone employed in the poison ivy study[11]. The trans amination product **10** is presumably formed via the iminoquinone intermediate **12** by the mechanism postulated by Corey et al. It is the reductive amination products (**11a-11d**)[14] which makes the ortho-quinone **3** unique in its behavior towards amines. The origin of these products may well be from the reduction of iminoquinone **12** by either the hydroquinone **2**, or the amine. In a pilot experiment we had observed that aminophenol **10** was capable of reducing **3** to hydroquinone **2**. Thus it is conceivable that **2** generated in-situ in these reactions could undergo a redox reaction with **12**. It should be noted that in the benzylamine reaction (entry 6) **2** was isolated in 30% yield. Alternatively,generation of **11** can be rationalized in an analogous fashion (i.e. oxidation of amine by **12**) to the studies by Itoh and co-workers[15] on the reaction of amines with the trimethyl ester of coenzyme PQQ. ***To our knowledge this is the first demonstration of etoposide ortho-quinone 3 undergoing both the reductive and trans amination reactions with amines.***

With these results in hand, the reactions of thiols with **2** were examined as a working model to predict the reactivity pattern of **3** in vivo with thiol bearing proteins and intracellular glutathione. Table 2 summarizes the results of our study.

TABLE 2. REACTION OF 3 WITH THIOLS



13 2

Entry	Thiol (R=)	Rx Conditions	Products† (% yield)
1	-CH_2CH_2NHAc (excess)	MeOH/0.5 hr	13a(22):2(25)
2	-CH_2COOEt (1.1 equiv.)	THF/DMAP/17 hrs	13b(10):2‡
3	-Et(1.1 equiv.)	THF/DMAP/1 hr	13c(10):2‡
4	Cysteine (2 equiv.)	CH_3CN/0.5 hr	2(65)#
5*	Glutathione (2 equiv.)	MeOH/pH7/5 hr	2(36)#

†. Isolated yields ‡. not isolated but observed (TLC, H^1N.M.R.) as a major product. *. In this reaction Glutathione disulfide was isolated and fully characterized. #. No adducts of amine reaction observed.

These reactions were carried out under anaerobic conditions and at ambient temperature. Unlike the amine cases, reactions of **3** with thiols were relatively clean and somewhat predictable based on the documented studies.[11,16] Thiols usually not encontered in the biological system yielded both the Michael adduct (e.g **13**) and redox products (eg. **2**) as evidenced by entries 1-3. In contrast, natural thiols cysteine and glutathione (entry 4 and 5 respectively) quantitatively (by TLC) form the the redox products with no detectable evidence (H^1NMR) of thiol Michael adduct or amine reaction products.

In conclusion, we have demonstrated that etoposide ortho-quinone 3 is a powerful oxidant capable of undergoing a variety of reactions with nucleophiles. In particular, the in vivo biological ramifications of the reductive (alkylation) and trans amination reactions by the ortho-quinone metabolite of etoposide may be significant. For example,the formation of covalent adducts with the amino moiety of lysines contained in enzymes or vital proteins could result in inhibition of vital cellular and or enzymatic functions. Also the trans amination process which results in the generation of an aldehyde could prove detrimental to cellular processes. In this light, the potential for **3** as a contributor towards the in vivo antitumor effect of etoposide is real and should not be discounted. Further indepth quantitative study with biological relevent nucleophiles, reaction conditions and concentrations is the basis of our future investigation.

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14. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their structures. Select 300 MHz NMR data ($CDCl_3$, δppm) form some compounds is included below: **11a.** 6.79 (s,1H), 6.55 (s,1H), 6.14 (s,1H), 5.96 (d,J=2.7Hz,2H), 5.81 (s,1H), 4.89 (d,J=3.3 Hz,1H), 4.73 (ABQ,JAB=4.9Hz,1H), 4.63 (d,J=7.6Hz,1H), 4.55 (m,1H), 4.39 (t,J= 8.5Hz,1H), 4.16 (m,2H), 3.74 (s,3H), 3.75-3.73 (m,1H), 3.56 (t,J=12.0Hz,1H), 3.42 (t,J=7.9Hz,1H), 3.33 (m,2H), 3.22 (dd,J=5.1,15.0Hz,1H), 2.98 (m,1H), 2.68 (s,3H), 1.60 (bs,1H), 1.38 (d, J=4.83Hz,3H). **11b.** 6.78 (s,1H), 6.52 (s,1H), 6.13 (s,1H), 5.94 (d,J=2.6Hz,2H), 5.76 (s,1H), 4.86 (d, J=3.3Hz,1H), 4.72 (ABQ,J=4.9Hz,1H), 4.62(d,J=7.6Hz,1H), 4.51 (d,J=4.9Hz,1H), 4.37(t,J=8.4Hz,1H), 4.16 (m,2H), 3.73 (s,3H), 3.73 (m,1H), 3.55(t,J=12.1Hz,1H), 3.41 (t,J=7.6Hz,1H), 3.32 (m,2H), 3.20 (dd,J=5.1,14.9Hz,1H), 2.91(m,3H), 2.72 (bs,OH), 2.52 (bs,OH), 1.55-1.25 (m,4H), 1.36 (d, J=5.0Hz,3H), 0.86 (t,J=7.4Hz,3H). **11c.** 6.79 (s,1H), 6.53 (s,1H), 6.10 (s,1H), 5.93 (d,2H,J=2.37Hz), 5.73 (s,1H), 4.87 (bs,1H), 4.72 (dd,1H,J=9.63Hz), 4.59-4.56 (m,2H), 4.38 (t,1H,J=9.01Hz), 4.18-4.15 (m,2H), 3.76-3.66 (m,5H), 3.56 (t,1H,J=9.75Hz), 3.44-3.30 (m,6H), 2.94 (bs,2H), 1.86-1.58 (m,5H), 1.37 (d,3H,J=4.93Hz), 1.24-0.80(m,5H).
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