



**HAL**  
open science

# Synthesis and biological evaluation of DIDS analogues as efficient inhibitors of RAD51 involved in homologous recombination

Alexandre Demeyer, Lucie Fonteneau, Marion Liennard, Claire Foyer, Pierre Weigel, Adèle Laurent, Jacques Lebreton, Fabrice Fleury, Monique Mathé-Allainmat

## ► To cite this version:

Alexandre Demeyer, Lucie Fonteneau, Marion Liennard, Claire Foyer, Pierre Weigel, et al.. Synthesis and biological evaluation of DIDS analogues as efficient inhibitors of RAD51 involved in homologous recombination. *Bioorganic and Medicinal Chemistry Letters*, 2023, 87, pp.129261. 10.1016/j.bmcl.2023.129261 . hal-04234850

**HAL Id: hal-04234850**

**<https://hal.science/hal-04234850>**

Submitted on 10 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Public Domain

# Synthesis and Biological Evaluation of DIDS Analogues as Efficient Inhibitors of RAD51 Involved in Homologous Recombination

Alexandre Demeyer,<sup>†1</sup> Lucie Fonteneau,<sup>†1</sup> Marion Liennard,<sup>† ‡</sup>, Claire Foyer,<sup>‡</sup> Pierre Weigel,<sup>†</sup> Adèle Laurent,<sup>‡</sup> Jacques Lebreton,<sup>‡</sup> Fabrice Fleury<sup>\*†</sup> and Monique Mathé-Allainmat<sup>\*‡</sup>

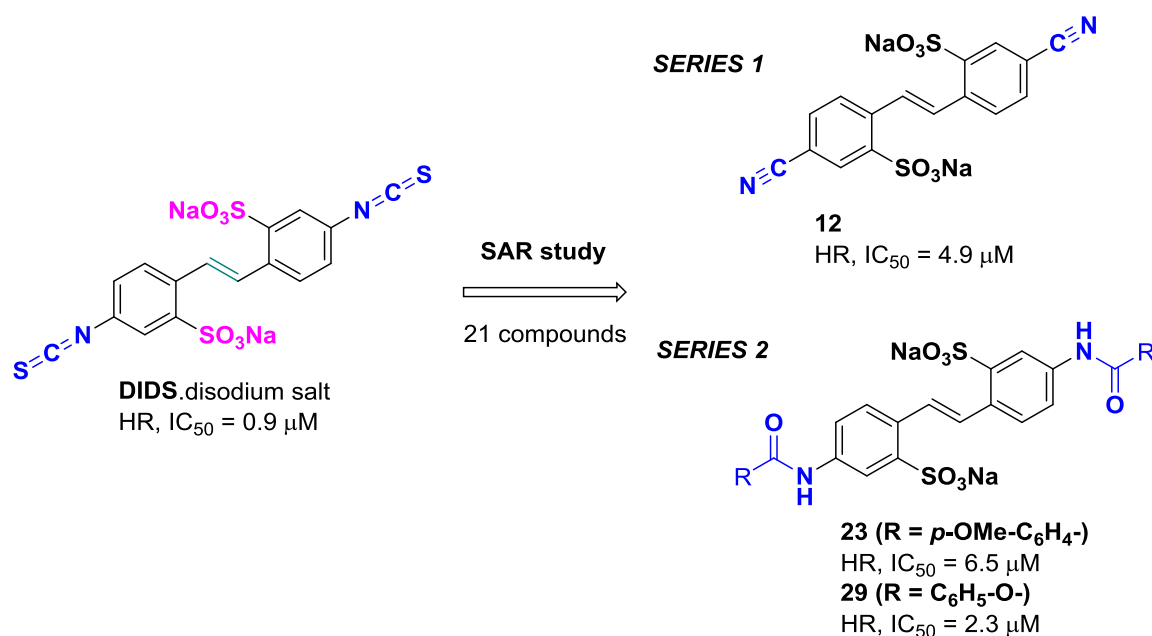
<sup>(†)</sup> Nantes Université, CNRS, US2B, UMR 6286, DNA-repair group, F-44000 Nantes, France.

<sup>(‡)</sup> Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France.

## Abstract

RAD51 is a pivotal protein of the homologous recombination DNA repair pathway, and is overexpressed in some cancer cells, disrupting then the efficiency of cancer-treatments. The development of RAD51 inhibitors appears as a promising solution to restore these cancer cells sensitization to radio- or chemotherapy. From a small molecule identified as a modulator of RAD51, the 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), two series of analogues with small or bulky substituents on the aromatic parts of the stilbene moiety were prepared for a structure-activity relationship study. Three compounds, the cyano analogue (**12**), and benzamide (**23**) or phenylcarbamate (**29**) analogues of DIDS were characterized as novel potent RAD51 inhibitors with HR inhibition in the micromolar range.

## Graphical abstract



28 **Key words :** Homologous recombination, DNA repair, RAD51, stilbene 2,2'-disulfonic  
29 compounds, inhibitor.

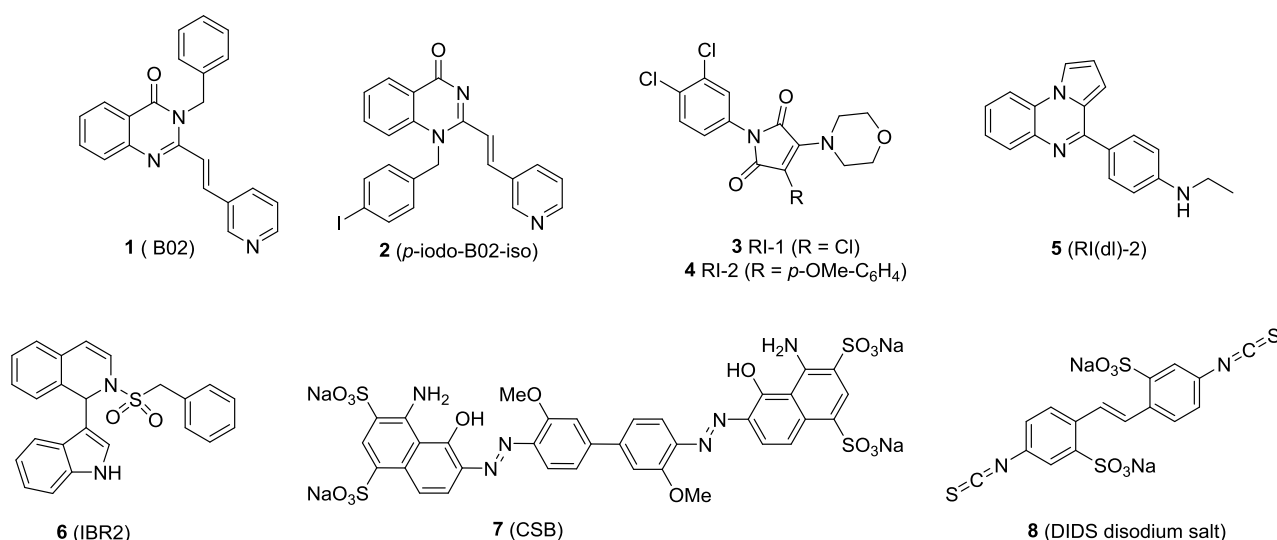
30

31 DNA repair process is particularly important in maintaining genomic integrity. In  
32 proliferative diseases therapies such as radiotherapy or chemotherapy, this process can restore  
33 the DNA damaged with the treatment, and induce a radio- or chemo-resistance. Homologous  
34 recombination (HR) is one of the two cellular pathways that repair DNA double-strand breaks  
35 (DSBs) [1]. It is catalyzed with RAD51 a highly conserved protein overexpressed in a wide  
36 variety of cancers and consequently contributing to drug resistance [2]. Elevated level of  
37 RAD51 mRNA is also associated with higher risk of tumor relapse and metastases [3]. It is to  
38 be noted that currently cancer treatment for patients with deficiency in HR-DNA repair  
39 systems are accessible with small molecules such as PARP inhibitors [4]. RAD51 is also  
40 investigated for its clinical utility as a biomarker [5] for the selection of patients with HR  
41 deficient tumors [6] or for prognosis of cellular DNA damage induced by chemotherapy [7].  
42 RAD51 disrupting DNA repair by HR either indirectly or directly is also clinically relevant  
43 especially to improve response to radio- or chemotherapy treatment.

44 During the last decade, extended studies have been done with RAD51 inhibitors  
45 including peptides, nucleic acid and small synthetic molecules. Peptide inhibitors have been  
46 generated to modulate the interaction between RAD51 monomer-monomer. Several peptide  
47 sequences from BRC motifs of BRCA2 have shown efficiency to dissociate the  
48 nucleofilament of RAD51 by inhibiting the auto-association of RAD51 on the DNA.[8] DNA  
49 aptamers (A13, 130 or A79) were identified by Martinez and collaborators as direct  
50 modulators of RAD51 and their inhibitory effect impacted nucleofilament formation rather  
51 than dissociation, inducing so the stability of ATP/RAD51/ssDNA complex [9]. Small  
52 molecular weight molecules are also required to interfere with RAD51 protein to disrupt  
53 either the polymerization and heptamer formation process or the binding of RAD51 to ssDNA  
54 during nucleofilament formation [1] [10]. Most of these molecules have been identified by  
55 high throughput screening of chemical compounds libraries. From the NIH Small Molecule  
56 Repository (>20 000 compounds), Fei Huang and collaborators [11] identified a compound  
57 B02 (Figure 1, compound 1) which selectively inhibited hRAD51 with an IC<sub>50</sub> of 27.4 μM on  
58 a D-loop assay. They recently published a series of B02 isomers with significant higher  
59 efficiency in inhibition of HR in human cells (Figure 1, compound 2). [12] Mediated by DNA  
60 recombinase RAD51, B02 was also proved to sensitize malaria parasites to chemotherapeutics

61 such as artemisinin and chloroquine [13]. With a similar virtual approach Connell and  
 62 collaborators identified RI-1 (Figure 1, compound **3**) that disrupted HR with an IC<sub>50</sub> of 20  
 63 μM. Due to its reactivity as a Michael acceptor [14] inducing an irreversible profile, chemical  
 64 optimization of RI-1 was done and substitution of the chloro substituent with a p-  
 65 methoxyphenyl group afforded a compound **4** (Figure 1, RI-2) with lower activity but  
 66 reversible binding.[15] The same authors applied two parallel screening of more than 8,000  
 67 compounds on RAD51-ssDNA binding assay and RAD51-dependent D-loop assay, in order  
 68 to identify selective inhibitors. After chemical optimization of a promising lead, compound **5**  
 69 (Figure 1, RI (dl)-2) was found to displayed improved D-loop activity, without detectable  
 70 effect on RAD51-ssDNA binding.[16] From Nanosyn libraries (24,000 compounds) a  
 71 sulfonamide synthetic alkaloid **6** (Figure 1, IBR2) was confirmed by SPR to bind RAD51 and  
 72 to inhibit HR by disrupting RAD51 heptamers inducing filament formation.[17] From a  
 73 library of 1120 compounds, Chicago Sky Blue (CSB), a dye molecule was revealed as  
 74 RAD51 inhibitor in the low nanomolar range (Figure 1, compound **7**).[18] Screening  
 75 known biological compounds and drugs, on RAD51 strand-exchange assay *in vitro*,  
 76 Kurumizaka's team identified a disulfonic compound 4,4'-diisothiocyanostilbene-2,2'-  
 77 disulfonic acid **8** (DIDS), with 50 % effect around 1-2 μM.[19]

78



79  
80

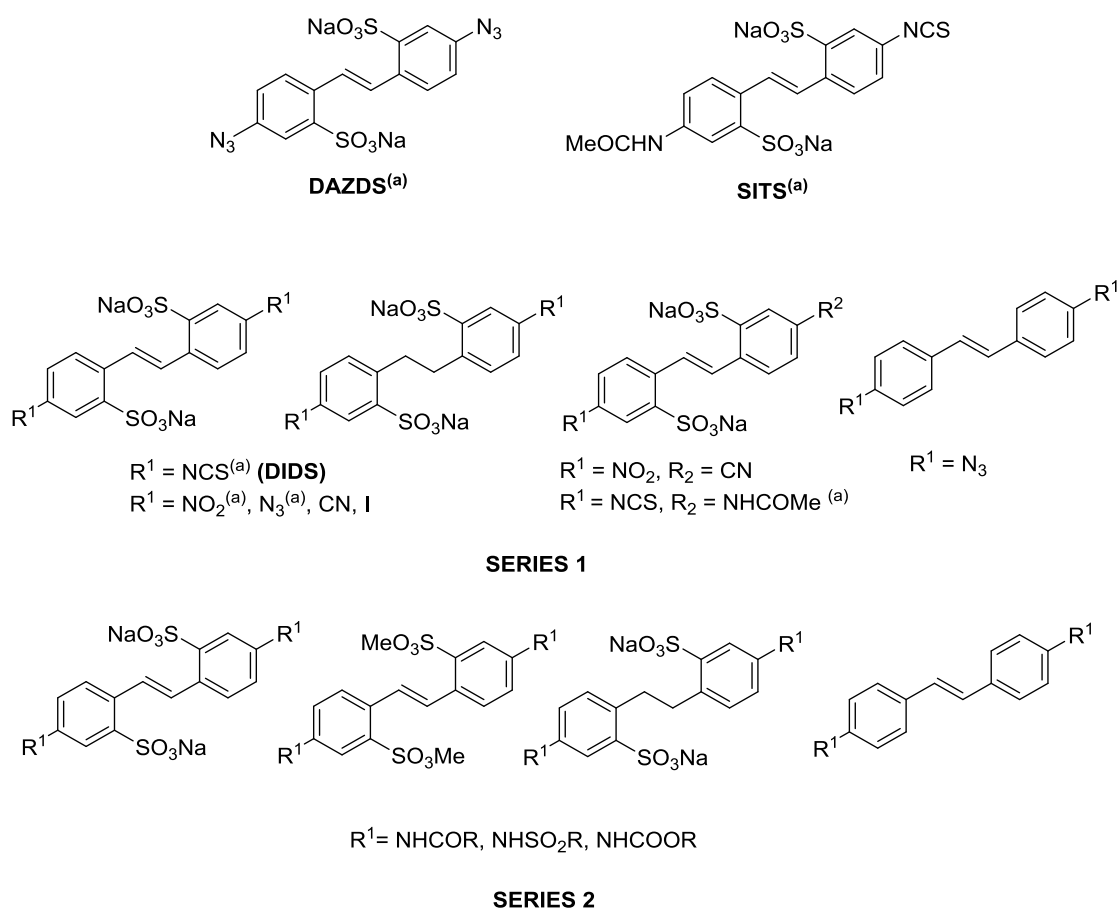
81 **Fig 1.** Small (hetero)aromatic molecules, modulators of the HR process.

82

83 Although it seemed that RAD51 inhibition resulted from DIDS direct interaction with  
 84 RAD51 protein, DIDS mechanism of action was not clearly elucidated. Moreover, DIDS

85 suffered from limitations which impair its potential use against cancer, regarding for example  
 86 selectivity over other biological targets or chemical stability of the molecule. Especially,  
 87 DIDS as inhibitor is not specific of the RAD51 protein. It also inhibits membrane transporters  
 88 [20] or anion channels [21], and its isothiocyanate functions may be responsible of some of  
 89 these activities. [22] As a chemical weakness, DIDS stability in water has been discussed  
 90 [23], and the potential hydrolysis of the isothiocyanate function could afford DIDS oligomers  
 91 also identified as chloride channel blockers. [24]

92



93

94 **Fig. 2** : Commercial <sup>(a)</sup> and synthetic DIDS analogues studied as RAD51 potential inhibitors.

95

96 In a previous work, we selected four commercial analogues of DIDS and showed that  
 97 DAZDS and SITS (Figure 2) were also able to prevent the formation of D-loop with an IC<sub>50</sub>  
 98 of 44.3 μM and 29 μM respectively, compared to 0.9 μM for the DIDS [25].

99 In this work, we have performed the synthesis of two novel series of DIDS analogues as  
 100 potential RAD51 inhibitors. Thereby chemical modifications were introduced on three

101 distinct positions of the 4,4'-substituted-2,2'-disulfonic stilbene scaffold for a structure-  
102 activity relationship study, in order to identify a good substitute to DIDS.

103 The compounds were tested *in vitro*, firstly for their effect on the invasion DNA (D-loop  
104 assay) and DNA strand exchange steps of RAD51 activity and secondly for their cytotoxicity  
105 effect on cancer cell lines overexpressing RAD51.

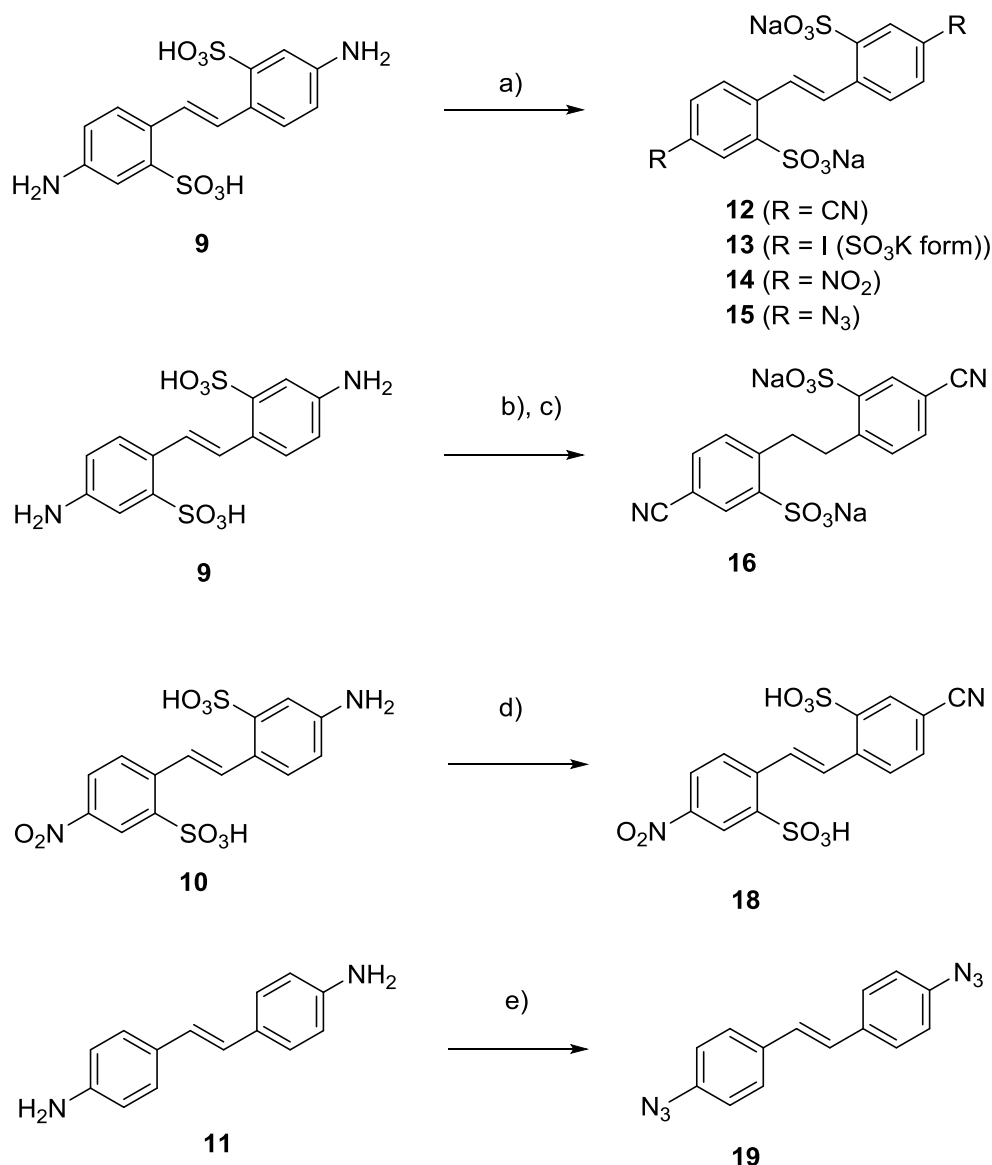
106 Two series of DIDS analogues have been synthesized, the first one including  
107 compounds bearing small chemical groups in place of the isothiocyanate group (NCS) in  
108 DIDS (Figure 2, series 1) and the second one derived from 4,4'-diamino analogue of DIDS  
109 (DADS) to develop compounds such as amide, sulfonamide and carbamate analogues of  
110 DIDS (Figure 2, series 2). These functions are known to be chemically stable in neutral  
111 conditions compared to the isothiocyanate one and nitrile or carbamate groups are also key  
112 structural motifs of many natural or synthetic drugs.[26, 27] To complete the SAR study,  
113 molecules with methyl sulfonate ester function or lacking the sulfonic acid function as well as  
114 the unsaturated ethylenic chain, have also been prepared, in order to determine the impact of  
115 these modifications on the HR activity. These novel DIDS analogues were prepared from  
116 commercial compounds such as 4,4'-diamino-2,2'-stilbenedisulfonic acid **9** (DADS), its 4-  
117 nitro-4'-amino analogue **10** and the 4,4'-diamino stilbene **11**.

118 Starting from diamino or monoamino stilbene compounds **9**, **10** or **11**, small  
119 substituents such as cyano, iodo or azido substituents could be efficiently introduced using  
120 classical Sandmeyer conditions (Scheme 1). The targeted compounds **12-13**, **18** and **19** were  
121 obtained with moderate to good yield (44 to 90 %) after purification by simple precipitation in  
122 a mixture of MeOH/Et<sub>2</sub>O. To access to the unsaturated cyano analogue **16**, reduction of the  
123 double bond of DADS to give the corresponding 4,4'-diaminodibenzyl-2,2'-disulfonic acid  
124 (DADB) was performed in basic conditions using Raney Nickel as the catalyst [28], prior to  
125 the Sandmeyer reaction.

126

127

128



129

130 **Scheme 1. Synthesis of DIDS analogues of series 1** : Reagents and conditions: a) NaNO<sub>2</sub> (2  
 131 equiv), AcOH (5 equiv), NaCN (4 equiv), CuCl (0.25 equiv), H<sub>2</sub>O, rt, 2 d, 67% for **12**;  
 132 NaNO<sub>2</sub> (2 equiv), AcOH (5 equiv) KI (5 equiv), CuI (0.1 equiv), H<sub>2</sub>O, rt, 2 d, 47% for **13**; **14**  
 133 and **15** are commercial compounds; b) RaNi, [H<sub>2</sub>] 1 atm., NaOH (2 equiv), H<sub>2</sub>O, 50 °C, 2 d,  
 134 74% of DADB; c) DADB, NaNO<sub>2</sub> (2 equiv), AcOH (5 equiv), NaCN (4 equiv), CuCl (0.25  
 135 equiv) water, rt, 3 d, 44% for **16**; d) NaNO<sub>2</sub> (1 equiv), AcOH (3 equiv), NaCN (2 equiv),  
 136 CuCl (0.5 equiv), H<sub>2</sub>O, rt, 1.5 d, 71%; e) NaNO<sub>2</sub> (2 equiv), AcOH (5 equiv), NaN<sub>3</sub> (2 equiv),  
 137 H<sub>2</sub>O, rt, 3 h, 90% .  
 138

138

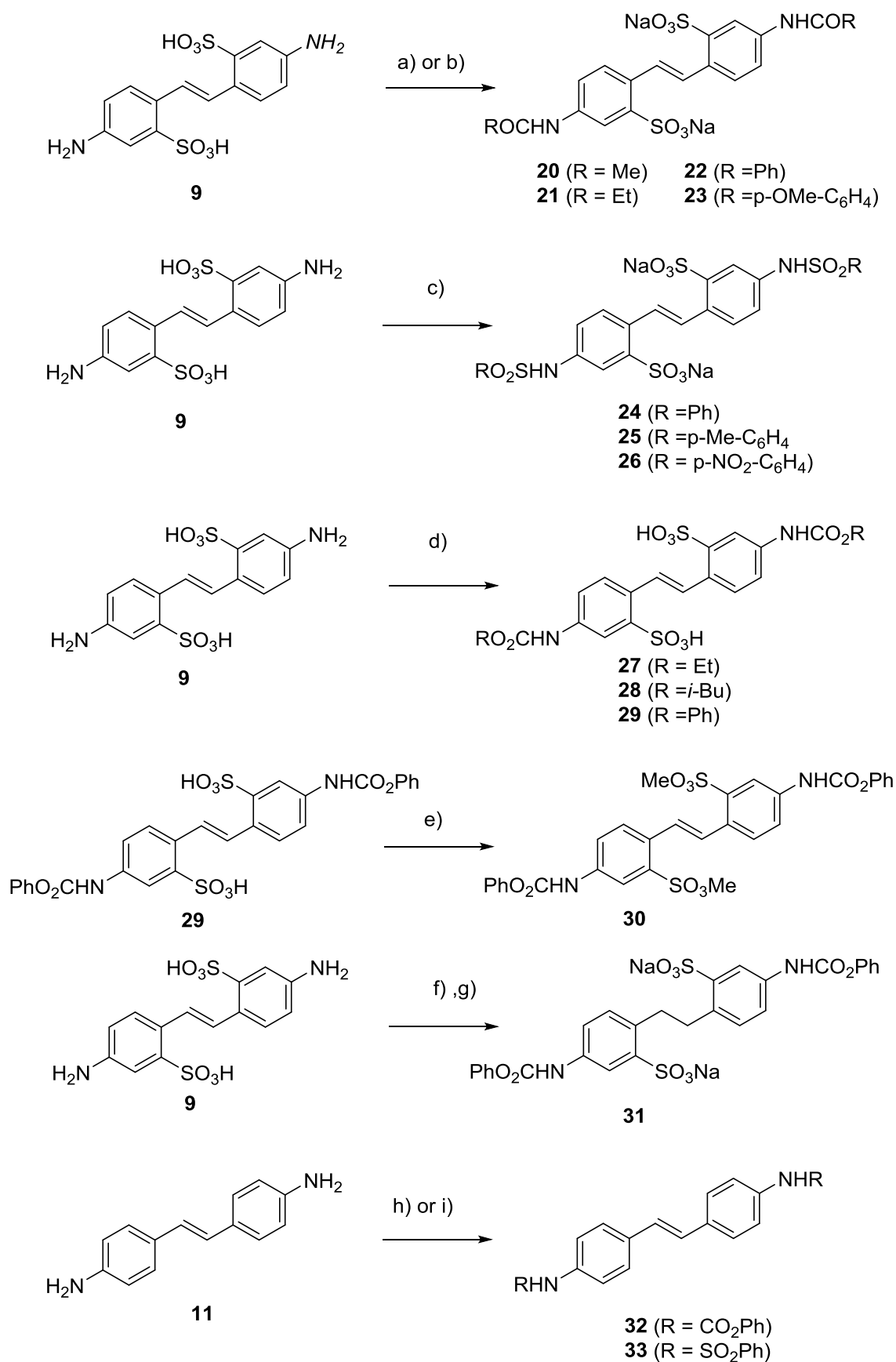
139

139 The second series of DIDS analogues, compounds **20-29** and **32-33**, was prepared by  
 140 acylation or sulfonylation of the amino precursors **9** or **11** (Scheme 2). In basic aqueous  
 141 conditions using anhydrides, sulfonyl chlorides or chloroformates as the reagents, the amides  
 142 **20-22**, the sulfonamides **24-26** or the carbamates **27-29** were obtained with good yields (62 to  
 143 85 %) after purification by simple precipitation. With acyl chlorides as the acylating agents,  
 144 reactions were performed in DMF using triethylamine as the base, and the amide **23** was

145 obtained with a moderate yield of 44 %. To determine the rule of the sulfonic function on the  
146 activity profile, esterification of the phenyl carbamate analogue **29** was achieved by refluxing  
147 its acidic form in toluene in the presence of an excess of trimethyl orthoacetate.[29] [30] The  
148 methyl sulfonate derivative **30** was obtained with 32 % yield. The sodium salt of DADB  
149 (reduced DADS) was also acylated with an excess of phenyl chloroformate in aqueous  
150 medium (H<sub>2</sub>O/dioxane) to give compound **31** with 58 % yield. It is to be noted that some  
151 attempt to reduce the double bond of compound **29** with NaBH<sub>4</sub> and Raney Nickel [31] to  
152 afford **31** in a single step, didn't work. Finally diamino stilbene **11** was reacted with phenyl  
153 chloroformate or phenyl sulfonyl chloride in dichloromethane, in the presence of an organic  
154 base, to give respectively carbamate **32** (54 % yield) and sulfonamide **33** (41 % yield).

155





156

157 **Scheme 2. Synthesis of DIDS analogues of series 2** : Reagents and conditions: a)  
 158 ROCOCOR (4 eq.), Na<sub>2</sub>CO<sub>3</sub> (5 eq.), nBu<sub>4</sub>NCl (0.1 eq.), H<sub>2</sub>O/dioxane (5:1), 2h, 62 % for **20**

159 (R = Me); overnight, 76 % for **21** (R = Et); overnight, 70 % for **22** (R = C<sub>6</sub>H<sub>5</sub>); b) RCOCl (4  
160 eq.), DIEA (5 eq.), DMF, 80 °C, 4 h, 44 % for **23** (R = p-OMe-C<sub>6</sub>H<sub>4</sub>); c) RSO<sub>2</sub>Cl (2.5 eq.),  
161 Na<sub>2</sub>CO<sub>3</sub> (5 eq.), H<sub>2</sub>O/dioxane (5:1), rt, overnight, 61 % for **24** (R = C<sub>6</sub>H<sub>5</sub>); 87 % for **25** (R =  
162 p-Me-C<sub>6</sub>H<sub>4</sub>); 81 % for **26** (R = p-NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>); d) ROCOCl (2.5 eq.), Na<sub>2</sub>CO<sub>3</sub> (5 eq.),  
163 H<sub>2</sub>O/dioxane (5:1), rt, overnight, 66 % for **27** (R = Et); 67 % for **28** (R = *iso*Bu); 90 % for **29**  
164 (R = C<sub>6</sub>H<sub>5</sub>); e) CH<sub>3</sub>C(OMe)<sub>3</sub> (4 eq.), toluene, reflux, 17 h, 32 %; f) Ra-Ni, [H<sub>2</sub>] 1 atm., NaOH  
165 (2 equiv), H<sub>2</sub>O, 50 °C, 2 d, 74% of DADB; g) PhOCOCl (4 eq.), H<sub>2</sub>O/dioxane (3:1),  
166 overnight, 58 %; h) PhOCOCl (4 eq.), DIEA (6 eq.), CH<sub>2</sub>Cl<sub>2</sub>, rt, 24 h, 54 % for **32**; i )  
167 RSO<sub>2</sub>Cl (2.2 eq.), Et<sub>3</sub>N (4 eq.), CH<sub>2</sub>Cl<sub>2</sub>, rt, 24 h, 41 % for **33**.

168  
169 With these as-prepared DIDS analogues in hands, we next focused our attention to evaluate  
170 them as potential RAD51 inhibitors with two *in vitro* activity tests developed to identify  
171 strand exchange activity. The first bioassay is an electrophoresis-based DNA strand exchange  
172 [18] between a labeled short single strand ssDNA (58-ssDNA\*) and a homologous double  
173 strand ds-DNA (33-ds-DNA) catalyzed by RAD51. Using polyacrylamide gel electrophoresis  
174 the formation of a novel dsDNA formed (58/33-dsDNA\*) could be measured in a dose-  
175 dependent manner. The second bioassay is a DNA displacement loop called D-loop [32] using  
176 labeled 100-ss-DNA\*\*.

177 The compounds of series 1 and series 2 were evaluated in one or both biochemical assays and  
178 compared to DIDS which gave similar results in the electrophoresis-based DNA exchange  
179 assay or the D-loop assay, and IC<sub>50</sub> values of 0.75 μM and 0.9 μM respectively. We could  
180 observe that substitution of the isothiocyanate function (NCS) with a strongly electro-  
181 withdrawing nitro group (compound **14**) dramatically decreased the effect up to 50 fold. A  
182 bulky polarizing substituent such as a iodo substituent (compound **13**, IC<sub>50</sub> = 34 μM) as well  
183 as the azido function (compound **15**, IC<sub>50</sub> = 21.5 μM) were less deleterious, but the sulfonate  
184 functions seemed to be crucial as the activity of the azido compound **19** devoid of these  
185 functions decreased with an IC<sub>50</sub> value up to 50 μM. Our best compound in this first series  
186 was compound **12**, with cyano substituents in place of the isothiocyanate ones and giving  
187 RAD51 activity inhibition with an IC<sub>50</sub> value of 4.9 μM. Furthermore in this series,  
188 unsymmetrical DIDS analogues bearing either one NCS group such as the commercial  
189 acetamido compound **17**, or one CN function such as the nitro compound **18** conserved  
190 RAD51 activity, but in the range of 30 micromolar, six fold higher than our best di-cyano  
191 compound **12**. Finally reduction of the double bond of dicyano compound **12**, to give the  
192 flexible 1,2-diphenyl ethane analogue **16**, induced loss of RAD51 activity.

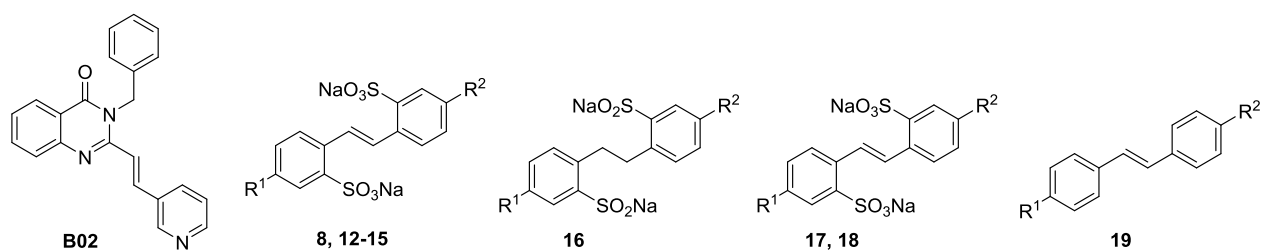
193

194

195 **Table 1** : RAD51 activity evaluation for compounds of series 1

196

197  
198

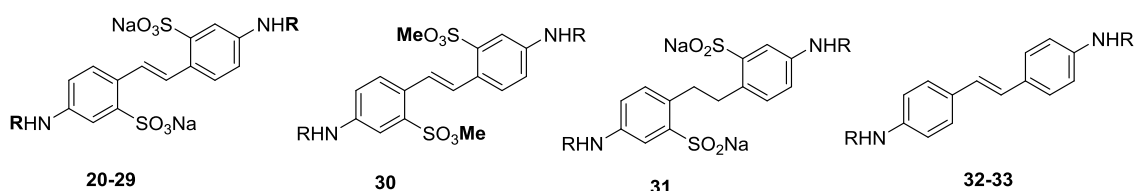


Compound	R <sup>1</sup>	R <sup>2</sup>	IC <sub>50</sub> (μM) (DNA strand exchange assay)	IC <sub>50</sub> (μM) (D-loop)
<b>B02</b>	-	-	-	27.4 <sup>b</sup>
<b>8<sup>a</sup></b> (DIDS)	NCS	NCS	<b>0.75</b>	<b>0.9</b>
<b>12</b>	CN	CN	<b>1.3</b>	<b>4.9</b>
<b>13</b>	I	I	<b>17.5</b>	34
<b>14<sup>a</sup></b>	NO <sub>2</sub>	NO <sub>2</sub>	-	>50
<b>15<sup>a</sup></b> (DAZDS)	N <sub>3</sub>	N <sub>3</sub>	<b>10</b>	21.5
<b>16</b>	CN	CN	-	>50
<b>17<sup>a</sup></b> (SITS)	NCS	NHCOMe	-	29
<b>18</b>	CN	NO <sub>2</sub>	-	20
<b>19</b>	N <sub>3</sub>	N <sub>3</sub>	-	>50

199 <sup>a)</sup>commercial compound; <sup>b)</sup> ref 11

200 For the second series with bulky amido, sulfonamide or carbonate residues in place of the  
 201 NCS one, only the residues with aromatic moieties, such as compounds **23**, **25**, **29**, **31** and **32**,  
 202 gave IC<sub>50</sub> values in the range of 2.3 μM to 30 μM. Sulfonamide compounds **24-26**, with an  
 203 acidic NH proton, were less efficient than amide (compound **23**) or carbonate (compound **29**)  
 204 analogues. The best compound in this second series was the diphenylcarbonate **29**, giving  
 205 similar results in the electrophoresis-based DNA exchange assay and the D-loop assay, with  
 206 IC<sub>50</sub> values of 3.8 μM and 2.3 μM respectively. We wanted to block the ionic character of  
 207 compound **29**, by esterification of the sulfonic functions. Satisfyingly we observed that the  
 208 bis-methyl sulfonate **30** was inactive, pointing out the crucial role of these free sulfonic  
 209 functions to block HR activity. Here also, reduction of the double bond to access to  
 210 compound **31**, or exclusion of the sulfonic functions (compound **32**) induced a 10-fold  
 211 decrease of the activity value, compared to the parent compound **29**.  
 212 If the double bond as well as the sulfonic functions of these stilbene compounds appeared as  
 213 important elements for RAD51 activity, complementary structure-activity conclusions still  
 214 remain difficult. Taking in account that the cyano functions in compound **12** from series 1,  
 215 and the benzamide or phenylcarbamate groups of compounds **23** and **29** from series 2, have  
 216 no similar physico-chemical properties, this could imply that each series has its specific  
 217 interactions in a same binding site, or that each series has its own binding site with RAD51.

218  
 219 **Table 2** : RAD51 activity evaluation for compounds of series 2



220

221

Compound	R	IC <sub>50</sub> (μM) (DNA strand exchange assay)	IC <sub>50</sub> (μM) (D-loop)
<b>8</b> (DIDS)	-	<b>0.75</b>	<b>0.9</b>
<b>20</b>	MeCO-	>50	-
<b>21</b>	EtCO-	>50	-

22	PhCO-	>50	-
23	<i>p</i> OMe-C <sub>6</sub> H <sub>4</sub> CO-	3.0	6.5
24	PhSO <sub>2</sub> -	-	41.3
25	<i>p</i> Me-C <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> -	-	29
26	<i>p</i> NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> -	-	>50
27	EtOCO-	>50	-
28	<i>i</i> BuOCO-	-	>50
29	PhOCO-	3.8	2.3
30	PhOCO-	-	>50
31	PhOCO-	-	≈ 30
32	PhOCO-	-	30.5
33	PhSO <sub>2</sub> -	-	>50

222

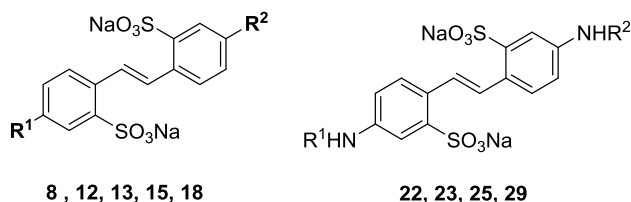
223

224 To envisage *in cellulo* evaluation of this family of molecules, the most interesting compounds  
 225 were selected and their cytotoxicity was evaluated on prostatic cancer cell (DU145) by the  
 226 MTT assay after 72h of exposure at 1μM, 10μM and 50μM (Table 3). The known  
 227 quinazolinone compound **B02**, showed a highly cytotoxic effect on DU145, reducing the  
 228 viability of DU145 from 21% to 91%, at the concentrations of 10μM and 50 μM,  
 229 respectively. In addition, DIDS poorly reduced cell viability from 10 to 17% in a dose-  
 230 dependent way. We could observe that most of the selected DIDS analogues of series 1 (**12**,  
 231 **13**, **15**, **18**) or of series 2 (**22**, **23**, **25**, **29**) had very low or no cytotoxic effect on DU145 cells

232 even at the high concentration of 50  $\mu\text{M}$ . Particularly, our most potent analogues such as di-  
 233 cyano compound **12**, and di-phenylcarbamate compound **29**, totally preserved cell viability at  
 234 concentrations of 1  $\mu\text{M}$  and 10  $\mu\text{M}$ . Cellular evaluation of our molecules alone have so shown  
 235 that in a context without DNA damage, where DNA repair pathways such as homologous  
 236 recombination are not activated, they have low effect on cell survival. In contrast, for DNA  
 237 damage response, preliminary results have shown that the molecules entered into the cell and  
 238 directly acted on RAD51-mediated HR pathway to sensitize DU145 cancer cell to cisplatin  
 239 treatment. [33]

240

241 **Table 3:** Viability of prostatic cancer cell DU145 in the presence of the selected compounds:



242

Compound	R <sup>1</sup>	R <sup>2</sup>	Cell Viability (% of control)		
			1 $\mu\text{M}$	10 $\mu\text{M}$	50 $\mu\text{M}$
<b>B02</b>	-		<b>95</b>	<b>79</b>	<b>9</b>
<b>8<sup>a</sup></b> (DIDS)	NCS	NCS	<b>90</b>	<b>87</b>	<b>83</b>
<b>12</b>	CN	CN	<b>99</b>	<b>94</b>	<b>100</b>
<b>13</b>	I	I	<b>91</b>	<b>89</b>	<b>84</b>
<b>15</b>	N <sub>3</sub>	N <sub>3</sub>	<b>87</b>	<b>94</b>	<b>93</b>
<b>18</b>	CN	NO <sub>2</sub>	<b>90</b>	<b>85</b>	<b>88</b>
<b>22</b>	PhCO-	PhCO-	<b>100</b>	<b>100</b>	<b>100</b>
<b>23</b>	<i>p</i> OMe-C <sub>6</sub> H <sub>4</sub> CO-	<i>p</i> OMe-C <sub>6</sub> H <sub>4</sub> CO-	-	-	<b>100</b>
<b>25</b>	<i>p</i> Me-C <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> -	<i>p</i> Me-C <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> -	<b>100</b>	<b>100</b>	<b>100</b>
<b>29</b>	PhOCO-	PhOCO-	<b>100</b>	<b>100</b>	<b>87</b>

243 <sup>a)</sup>commercial compound

244

245 In summary, in this work, we have developed analogues of DIDS, a known di-sulfonic  
246 stilbene compound identified as a HR potential inhibitor targeting specifically RAD51. Due to  
247 suspected chemical or biochemical instability of the isothiocyanate functions of DIDS, we  
248 have prepared two series of DIDS analogues bearing small or bulky substituents with various  
249 chemical properties, such as cyano, amide, sulfonamide or carbamate analogues. This  
250 preliminary structure-activity relationship has highlighted the crucial roles of the sulfonic acid  
251 functions as well as the double bond of the stilbene moiety for RAD51 activity. Three  
252 compounds, the di-cyano analogue **12**, the di-paramethoxybenzamide **23** and the di-  
253 phenylcarbamate analogue **29** with no cytotoxic effect on the selected cell line, have been  
254 characterized *in vitro* as potent compounds for inducing RAD51 activity inhibition. This  
255 encourage us to go further with *in cellulo* evaluations to ensure that the compounds target  
256 RAD51 in this HR pathway and could be proposed for cancer cells sensitization to  
257 chemotherapeutic treatment.

258

### 259 **Authors Information**

260 \* Corresponding authors, email addresses: [monique.mathe@univ-nantes.fr](mailto:monique.mathe@univ-nantes.fr) and  
261 [fabrice.fleury@univ-nantes.fr](mailto:fabrice.fleury@univ-nantes.fr)

262 <sup>1</sup> These authors contributed equally to this work.

263

### 264 **Notes**

265 The authors declare no competing financial interest.

266

### 267 **Aknowledgments**

268 This work was funded by CNRS, Nantes University and the “Région Pays-de Loire”  
269 (PIRAMID, <http://piramid-research.fr>) with a PhD thesis for A.D. and carried out with  
270 financial support from the SATT Ouest Valorisation in France. We thank CHEM-Symbiose  
271 platform (CEISAM laboratory) and IMPACT platform (US2B laboratory) for technical  
272 assistance.

273

### 274 **References**

275 [1] Ward A., Khanna K. K., Wiegman A. P. Targeting homologous recombination, new pre-  
276 clinical and clinical therapeutic combinations inhibiting RAD51. *Cancer Treat. Rev.* 2015;  
277 41:35–45. [doi.org/10.1016/j.ctrv.2014.10.006](https://doi.org/10.1016/j.ctrv.2014.10.006).

278  
279 [2] Leon- Galicia I., Diaz- Chavez J., Albino-Sanchez M.E., Garcia- Villa E.,  
280 Bermudez- Cruz R., Garcia- Mena J., Herrera L.A., García- Carrancá A., Gariglio P.  
281 Resveratrol decreases Rad51 expression and sensitizes cisplatin- resistant MCF- 7 breast  
282 cancer cells. *Oncol. Rep.* 2018; 39:3025-3033. doi: 10.3892/or.2018.6336.  
283  
284 [3] Barbano R., Copetti M., Perrone G., Paziienza V., Muscarella L. A., Balsamo T., Storlazzi  
285 C. T., Ripoli M., Rinaldi M., Valori V. M., Latiano T. P., Maiello E., Stanziale P., Carella M.,  
286 Mangia A., Pellegrini F., Bisceglia M., Onetti Muda A., Altomare V., Murgo R., Fazio V. M.,  
287 Parrella P. High RAD51 mRNA expression characterize estrogen receptor-  
288 positive/progesteron receptor-negative breast cancer and is associated with patient's outcome  
289 *Int. J. Cancer* 2011; 129(3):536–545. doi: 10.1002/ijc.25736  
290  
291 [4] Sachdev E., Tabatabai R., Roy V., Rimel B. J., Mita M. M. PARP Inhibition in Cancer:  
292 An Update on Clinical Development. *Target. Oncol.* 2019;14(6):657-679. doi:  
293 10.1007/s11523-019-00680-2.  
294  
295 [5] Gachechiladze M., Skarda J., Soltermann A., Joerger M. RAD51 as a potential surrogate  
296 marker for DNA repair capacity in solid malignancies. *Int. J. Cancer* 2017; 141(7):1286–  
297 1294. doi: 10.1002/ijc.30764.  
298  
299 [6] Graeser M., McCarthy A., Lord C. J., Savage K., Hills M., Salter J., Orr N., Parton M.;  
300 Smith I. E.; Reis-Filho J. S.; Dowsett M.; Ashworth A.; Turner N. C. A marker of  
301 homologous recombination predicts pathologic complete response to neoadjuvant  
302 chemotherapy in primary breast cancer. *Clin. Cancer Res.* 2010; 16(24):6159-6168. doi:  
303 10.1158/1078-0432.CCR-10-1027.  
304  
305 [7] Zhang X., Ma N., Yao W., Li S., Ren Z. RAD51 is a potential marker for prognosis and  
306 regulates cell proliferation in pancreatic cancer. *Cancer Cell Int.* 2019; 19:356.  
307 doi.org/10.1186/s12935-019-1077-6.  
308  
309 [8] Nomme J., Takizawa Y., Martinez S.F., Renodon-Cornière A., Fleury F., Weigel P.,  
310 Yamamoto K., Kurumizaka H., Takahashi M. Inhibition of filament formation of human  
311 Rad51 protein by a small peptide derived from the BRC-motif of the BRCA2 protein. *Genes*  
312 *Cells.* 2008; 13:471-484. doi: 10.1111/j.1365-2443.2008.01180.x.  
313  
314 [9] Martinez S. F., Renodon-Cornière A., Nomme J., Eveillard D., Fleury F., Takahashi M.,  
315 Weigel P. Targeting human Rad51 by specific DNA aptamers induces inhibition of  
316 homologous recombination. *Biochimie* 2010; 92:1832- 1838.  
317 doi:10.1016/j.biochi.2010.08.006.  
318  
319 [10] Budke B., Lv W., Kozikowski A. P., Connell P. P. Recent Developments Using Small  
320 Molecules to Target RAD51: How to Best Modulate RAD51 for Anticancer Therapy?  
321 *ChemMedChem.* 2016; 11(22):2468–2473. doi:10.1002/cmdc.201600426..  
322  
323 [11] Huang F., Motlekar N.A., Burgwin C. M., Napper A. D., Diamond S. L., Mazin A.V.,  
324 Identification of specific inhibitors of human RAD51 recombinase using high-throughput  
325 screening. *ACS Chem. Biol.* 2011; 6(6):628–635. doi:10.1021/cb100428c.  
326



327 [12] Shkundina I. S., Gall A. A., Dick A., Cocklin S., Mazin A.V., New RAD51 Inhibitors to  
328 Target Homologous Recombination in Human Cells. *Genes* 2021; 12:920.  
329 doi.org/10.3390/genes12060920.  
330

331 [13] Vydyam P., Dutta, D., Sutram N., Bhattacharyya S., Bhattacharyya M. K., A small-  
332 molecule inhibitor of the DNA recombinase Rad51 from *Plasmodium falciparum* synergizes  
333 with the antimalarial drugs artemisinin and chloroquine. *J. Biol. Chem.* 2019; 294(20):8171–  
334 8183. doi 10.1074/jbc.RA118.005009.  
335

336 [14] Budke B., Logan H. L., Kalin J. H., Zelivianskaia, A. S., Cameron McGuire W., Miller  
337 L. L., Stark J. M., Kozikowski A. P., Bishop D. K., Connell P. P. RI-1: a chemical inhibitor of  
338 RAD51 that disrupts homologous recombination in human cells. *Nucleic Acids Res.* 2012;  
339 40(15):7347–7357. doi:10.1093/nar/gks353.  
340

341 [15] Budke B., Kalin J. H., Pawlowski M., Zelivianskaia A. S., Wu M., Kozikowski A. P.,  
342 Connell P. P. An optimized RAD51 inhibitor that disrupts homologous recombination without  
343 requiring Michael acceptor reactivity. *J. Med. Chem.* 2013 ; 56(1):254–263.  
344 dx.doi.org/10.1021/jm301565b.  
345

346 [16] Lv W., Pawlowski M., Kozikowski A. P., Connell P. P., Kozikowski A. P. Development  
347 of Small Molecules that Specifically Inhibit the D- loop Activity of RAD51. *J. Med. Chem.*  
348 2016; 59(10):4511-4525. doi: 10.1021/acs.jmedchem.5b01762.  
349

350 [17] Zhu J., Zhou L., Wu G., Konig H., Lin X., Li G., Qiu X-L., Chen C-F., Hu C-M.,  
351 Goldblatt E., Bhatia R., Chamberlin A. R., Chen P-L., Lee W-H. A novel small molecule  
352 RAD51 inactivator overcomes imatinib-resistance in chronic myeloid leukaemia. *EMBO Mol.*  
353 *Med* 2013; 5:353–365. doi 10.1002/emmm.201201760.  
354

355 [18] Normand A., Rivière E., Renodon-Cornière A. Identification and characterization of  
356 human Rad51 inhibitors by screening of an existing drug library. *Biochem. Pharmacol.* 2014;  
357 91(3):293–300. doi.org/10.1016/j.bcp.2014.07.033.  
358

359 [19] Ishida T., Takizawa Y., Kainuma T., Inoue J., Mikawa T., Shibata T., Suzuki H., Tashiro  
360 S., Kurumizaka H. DIDS, a chemical compound that inhibits RAD51-mediated homologous  
361 pairing and strand exchange. *Nucleic Acids Res.* 2009; 37(10):3367–3376.  
362 doi:10.1093/nar/gkp200.  
363

364 [20] Wilson M. C., Meredith D., Bunnun C., Sessions R. B., Halestrap A. P. Studies on the  
365 DIDS-binding site of monocarboxylate transporter 1 suggest a homology model of the open  
366 conformation and a plausible translocation cycle. *J. Biol. Chem.* 2009; 284(30):20011-20021.  
367 doi: 10.1074/jbc.M109.014217.  
368

369 [21] Bollmann E., Schreiber J. A., Rittera N., Peischara S., Tran Hoa H., Wünsch B.,  
370 Strücker T., Meuth S., Budde T., Strutz-Seebohm N., Seebohm G. 4,4'-Diisothiocyanato-  
371 2,2'-Stilbenedisulfonic Acid (DIDS) Modulates the Activity of KCNQ1/KCNE1 Channels by  
372 an Interaction with the Central Pore Region. *Cell Physiol Biochem* 2020; 54:321-332. doi:  
373 10.33594/000000222.  
374

375 [22] Park J., Han J-H., Myung S-H., Kim T-H. Isothiocyanate groups of 4,4'-  
376 diisothiocyanatostilbene-2,2'-disulfonate (DIDS) inhibit cell penetration of octa-arginine  
377 (R8)-fused peptides. *Peptide Sci.* 2020, 26(3):e3237. doi.org/10.1002/psc.3237.  
378

379 [23] Jaunet-Lahary T., Goupille A., Jacquemin D., Fleury F., Graton J., Laurent A.D. A Joint  
380 Theoretical and Experimental Study of the Behavior of the DIDS Inhibitor and its  
381 Derivatives. *ChemPhysChem* 2016, 17: 2434 – 244. dx.doi.org/10.1002/cphc.201600107.  
382

383 [24] Matulef K., Howery A. E., Tan L., Kobertz W. R., Du Bois, J., Maduke M. Discovery of  
384 Potent CLC Chloride Channel Inhibitors. *ACS Chemical Biology* 2008, 3(7):419-428. doi :  
385 10.1021/cb800083a  
386

387 [25] Velic D., Demeyer A., Peterlini T., Benhelli-Mokrani H., Mathé-Allainmat M.,  
388 Masson, J.-Y., Fleury F. Molecular Determinant of DIDS Analogs Targeting RAD51 Activity.  
389 *Molecules* 2021, 26:5460. doi.org/10.3390/molecules26185460  
390

391 [26] Gosh A. K., Brindidi M. Organic Carbamates in Drug Design and Medicinal Chemistry.  
392 *J. Med. Chem.* 2015, 58:2895–2940. doi: 10.1021/jm501371s.  
393

394 [27] Scotti C., Barlow J. W. Natural Products Containing the Nitrile Functional Group and  
395 Their Biological Activities. *Nat. Prod. Commun.* 2022, 17(5): 1–24. doi:  
396 10.1177/1934578X221099973.  
397

398 [28] Bazanova N., Kholodkova N. V., Gostikin V. P. Reduction of 4,4'-Dinitrostilbene-2,2'-  
399 disulfonic Acid with Hydrogen on Raney Nickel. *Russ. J. Appl. Chem.* 2002; 75(3):436-440.  
400 doi:10.1023/A:1016150906701  
401

402 [29] Wrobel J., Green D., Jetter J., Kao W., Rogers J., Pérez M. C., Hardenburg J., Deecher  
403 D. C., López F. J., Arey B. J., Shen E. S. Synthesis of (bis)Sulfonic Acid, (bis)Benzamides  
404 as Follicle-Stimulating Hormone (FSH) Antagonists. *Bioorg. Med. Chem.* 2002; 10:639–656.  
405 doi.org/10.1016/S0968-0896(01)00324-8.  
406

407 [30] Trujillo J. I., Gopalan A. S. Facile Esterification of Sulfonic Acids and Carboxylic Acids  
408 with Triethylorthoacetate. *Tet. Lett.* 1993; 34(46):7355-7358. doi.org/10.1016/S0040-  
409 4039(00)60124-7.  
410

411 [31] Krishna Rao G., Gowda N. B., Ramakrishna R. A. RaneyNickel-Catalyzed  
412 Hydrogenation of Unsaturated Carboxylic Acids with Sodium Borohydride in Water,  
413 *Synth. Commun.* 2012; 42(6):893-904. doi: 10.1080/00397911.2010.533239.  
414

415 [32] Liu J., Sneed J., Heyer W.-D. In vitro assays for DNA pairing and recombination-  
416 associated DNA synthesis. *Methods Mol. Biol.* 2011, 745:363–383. doi:10.1007/978-1-61779-  
417 129-1\_21.  
418

419 [33] Demeyer A., Fonteneau L., Liennard M., Weigel P., Lebreton J., Mathé-Allainmat M.,  
420 Fleury F. Unpublished results.  
421  
422  
423  
424  
425

426  
427  
428  
429  
430  
431  
432  
433  
  
434