Tetrahedron Letters 61 (2020) 152267

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Anticancer agent synthesis designed by artificial intelligence: $Pd(OAc)_2$-catalyzed one-pot preparation of biphenyls and its application to a concise synthesis of various diazofluorenes

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ARTICLE INFO

Article history:
Received 23 June 2020
Revised 14 July 2020
Accepted 16 July 2020
Available online 14 August 2020

Keywords:
Artificial intelligence
Antitumor agent
Biphenyl
Diazofluorene

ABSTRACT

We successfully synthesized the antitumor agent 1-methoxydiazofluorene via an artificial intelligence (AI)-proposed design. The pivotal biphenyl scaffold of 1-methoxydiazofluorene was constructed by applying $Pd(OAc)_2$-catalyzed cycloaromatization, a method developed by our group. AI-proposed functional group conversion of the biphenyl intermediate provided 9-diazo-1-methoxy-9*H*-fluorene, which inhibits the proliferation of HeLa cells, was achieved in reasonable chemical yield. In addition, various diazofluorenes were synthesized using the above protocol and their antitumor effects were evaluated. As a result, several novel diazofluorenes, which have a stronger cytotoxic activity than cisplatin against various human epithelial cancer cells, were found.

Introduction

Artificial intelligence (AI) is playing an increasingly significant role in many fields [1]. AI is being utilized in self-driving cars [2], humanoid robots [3], and to produce medical diagnoses [4], to name a few examples. It will eventually develop to a stage where machines will work as teammates with humans. How exactly will AI evolve in the future? How quickly will AI transform the way people live? While the answer to these questions cannot be known at present, what is clear is that the field of synthetic organic chemistry is lagging behind in transitioning to the use of AI. One of the major advantages of AI is its ability to make swift and accurate calculations in determining the next best move, and this trait is leveraged in the use of AI in games and competitions. Similarly, if the AI loses, it can be upgraded via feedback gained by the experience. These concepts can be used to apply AI in the field of synthetic organic chemistry, although additional layers of complication must be considered. For example, verification experiments are essential in order to evaluate synthetic routes proposed by AI, and there are a number of factors that determine the superiority or inferiority of AI-proposed synthetic routes. The total yield, number of steps, reaction safety, and toxicity of intermediates and byproducts are added to the evaluation of each synthetic route. Furthermore, the verification of experimental results significantly depends on the skill of the experimenters. All of these make the evaluation of AI programs more complex. It therefore takes a long time to complete the verification experiments of the proposed synthetic routes, and this has contributed to the delay in the development and utilization of AI in organic synthetic chemistry.

In our previous paper related to AI-proposed drug synthesis, we demonstrated that our AI program, SYNSUP [5], is a useful tool in synthetic organic chemistry [6]. The reaction conditions of the key reaction (**1** → **2**) were studied after tuning a part of the reaction substrate structure proposed by the SYNSUP program (Scheme 1). Although the reaction mechanism of the key step was different from the one originally proposed, we found a novel method for the stereoselective synthesis of *trans* β-lactams. The usefulness of the protocol was verified by the demonstration of the total synthesis of SCH 47949 (**3**), a cholesterol absorption inhibitor [7]. It is worth noting that the functional group conversions (**2** → **3**) in this synthesis were all identical with the transformational processes proposed by AI.

As part of an effort to demonstrate the versatility of AI-proposed target molecule synthesis, we herein report a $Pd(OAc)_2$-catalyzed one-pot preparation of biphenyls and its application to 9-diazo-1-methoxy-9*H*-fluorene (**4**) synthesis. 1-Methoxydiazo-fluo-

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https://doi.org/10.1016/j.tetlet.2020.152267
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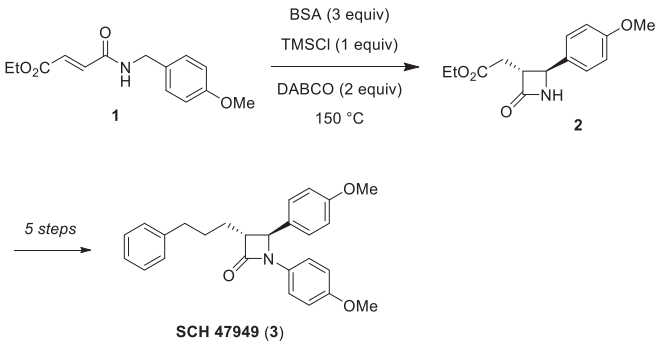
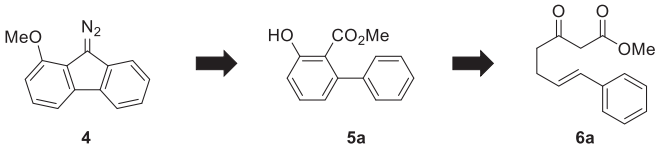
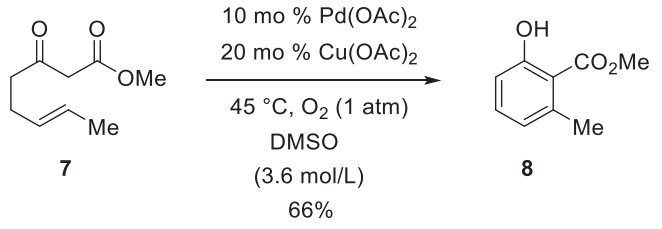


Scheme 1. AI-designed total synthesis of SCH 47949 (**3**).



Scheme 2. Retrosynthetic analysis of **4** proposed by SYNSUP.



Scheme 3. $Pd(OAc)_2$-catalyzed cycloaromatization.

rene **4**, a simple diazofluorene analog of kinamycins, inhibits the proliferation of HeLa cells [8].

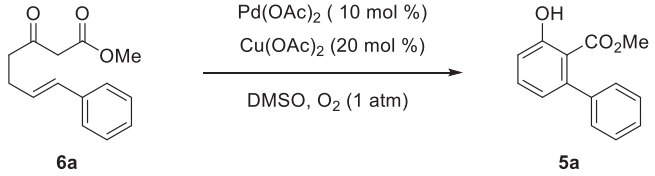
Scheme 2 shows the SYNSUP-proposed retrosynthetic analysis for 9-diazo-1-methoxy-9*H*-fluorene (**4**) (See Supporting Information-1). Namely, the target molecule **4** could be constructed through a series of functional group transformations of the biphenyl **5a**, which could be assembled to form the 1,3-dicarbonyl compound **6a** by the $Pd(OAc)_2$-catalyzed cycloaromatization (**7** → **8**) method recently developed by our group (Scheme 3) [9]. For example, methyl 2-hydroxy-6-methylbenzoate (**8**) was obtained catalytically from the acyclic unsaturated 3-keto ester **7** in a single step.

Results and discussion

First, we examined whether our cycloaromatization process could be adapted to use 1,3-dicarbonyl compound **6a** to assemble the target biphenyl compound **5a** [10]. The requisite substrate **6a** was easily synthesized in a reasonable chemical yield from cinnamyl chloride and dianion of methyl acetoacetate according to the standard procedure [11]. A number of different reaction parameters, such as concentration, temperature, and reaction time were tested to optimize this cycloaromatization.

Since **8** was obtained in a 66% yield by means of $Pd(OAc)_2$-catalyzed cycloaromatization (Scheme 3), we first applied the above reaction conditions to the present biphenyl synthesis. The catalyst and reoxidant were fixed, and different reaction temperatures, reaction times, and solvents were examined. Even at room temperature, the desired biphenyl **5a** was produced in a 20% yield; however, 65% of starting material **6a** was recovered (Table 1, entry 1). The catalytic reaction was performed at 45 °C for 12 h to afford **5a** (46%) together with **6a** (17%), and when this reaction time was extended to 18 h, the reaction gave **5a** in a 48% yield (Table 1, entries 2 and 3). Although **6a** was consumed completely, the conversion yield was not high. The ^1H NMR spectra of the crude product shows only **6a**, likely because some organic molecules were taken into the palladium complex. When this reaction was carried

Table 1
Evaluation of the reaction conditions for cycloaromatization.



6a **5a**

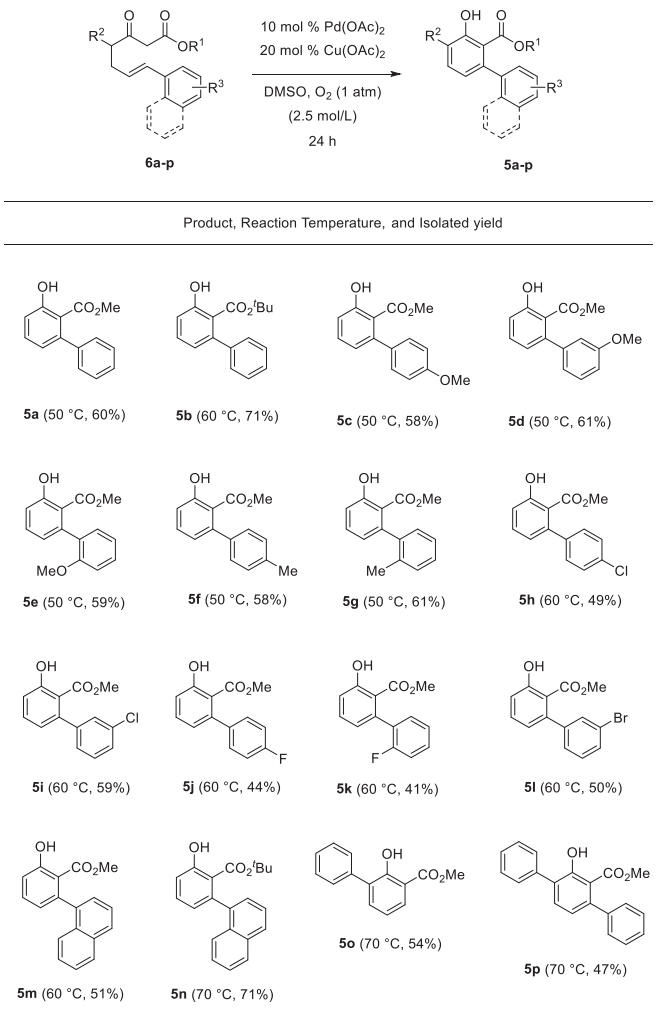
Entry	Temperature (°C)	Substrate concentration (mol/L)	Time (h)	Solvent	Yield (%)[a]
1	rt	3.6	24	DMSO	20 [65]
2	45	3.6	12	DMSO	46 [17]
3	45	3.6	18	DMSO	48
4	70	3.6	16	DMSO	29
5	45	3.6	18	toluene	28 [23]
6	45	3.6	18	DMF	34 [16]
7	45	3.6	18	1,2-DME	38 [30]
8	45	3.6	18	1,2-DCE	36 [31]
9[b]	45	3.6	18	DMSO	47
10[c]	45	3.6	18	DMSO	17
11	45	5.0	18	DMSO	45
12	50	2.5	24	DMSO	60
13[d]	50	2.5	24	DMSO	11
14[e]	50	2.5	24	DMSO	15

[a] Isolated yields in parentheses represent the recovered starting material **6a**.
[b] 10 mol % $Pd(TFA)_2$ was used instead of 10 mol % $Pd(OAc)_2$.
[c] 10 mol % $PdCl_2$ was used instead of 10 mol % of $Pd(OAc)_2$.
[d] N_2 was used instead of O_2.
[e] In the absence of 20 mol % $Cu(OAc)_2$.

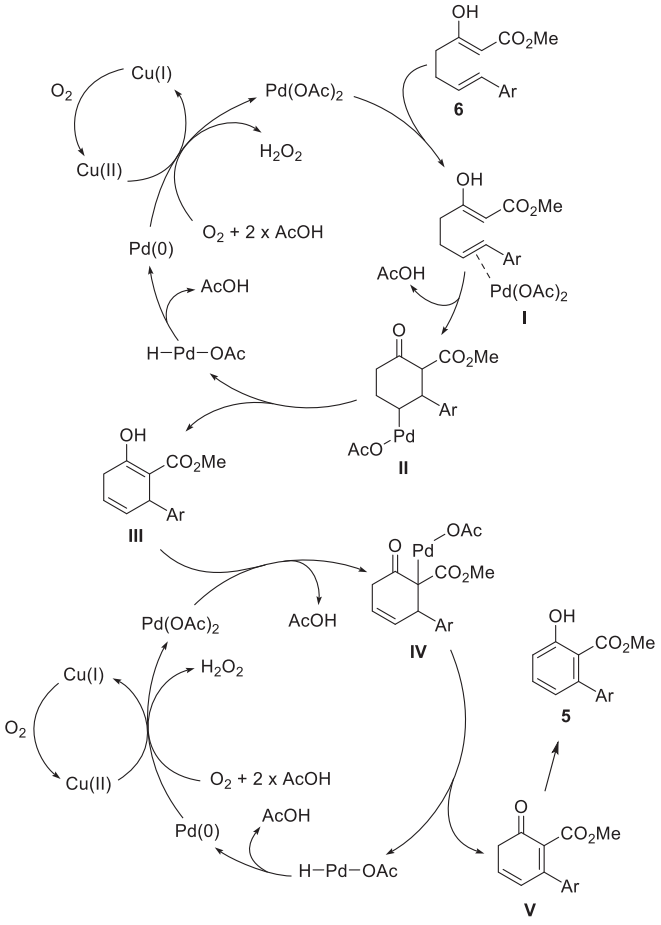
out at 70 °C, no other product was found; however, the isolation yield was drastically reduced to 29% (Table 1, entry 4). Next, the solvent effect was evaluated in this catalytic system. When this catalytic reaction was carried out using toluene, DMF, 1,2-dimethoxyethane (1,2-DME), or 1,2-dichloroethane (1,2-DCE) at 45 °C, a substantial amount of **6a** was recovered together with **5a** (Table 1, entries 5–8). When we examined the effect of the catalyst, $Pd(TFA)_2$ proved to be a useful catalyst; however, $PdCl_2$ was ineffective (Table 1, entries 9 and 10). Altering the reaction concentration resulted in a higher concentration (approximately 5.0 mol/L) of **6a** giving rise to **5a** in a 45% yield; however, when the reaction concentration was reduced using a 2.5 mol/L, the desired product **5a** was obtained with the highest yield (Table 1, entries 11 and 12). Finally, we found that when this reaction was carried out using nitrogen instead of oxygen, only 11% of **5a** was obtained, and only 15% of **5a** was obtained in the absence of $Cu(OAc)_2$. These results suggest that the Pd(0) generated in the system is oxidized smoothly to $Pd(OAc)_2$ only when an oxygen and $Cu(OAc)_2$ coexist (Table 1, entries 13 and 14).

To expand the versatility of this one-pot synthesis of biphenyls, the influence of different substituent groups on the benzene ring and side chain of **6** on the chemical yield was examined (Scheme 4). *tert*-Butyl ester **5b** was obtained in a 71% yield when the reaction was performed at 60 °C. Changing the substituent group on the benzene ring of reaction substrates **6c–e** to a methoxy substituent had little influence on the chemical yields of products **5c–e**. In the case of methyl-substituted substrates **5f–g**, the product yields were slightly decreased. If a halogen atom, such as chloride or fluoride, was placed as a substituent on the benzene ring of substrate **6**, the chemical yield decreased slightly. In the case of substrate **6k,** which is substituted by fluoride, the yield of **5k** decreased to 41%. The desired products were obtained in reasonable yields when using substrates **6m–n** with a bulky naphthyl group as a substituent. Interestingly, each cyclization of substrate **6o** with a phenyl group at the 4 position and substrate **6p** with a phenyl group at both the 4 and 7 positions gives biphenyl **5o** and terphenyl **5p** in moderate yields.

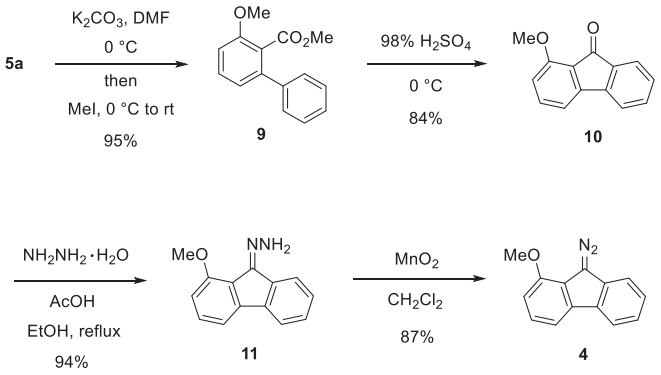
Scheme 5 shows a possible reaction mechanism of the one-pot preparation of biphenyls. After activation of an isolated olefin of **6** by $Pd(OAc)_2$, an enolate carbon in **I** attacks the terminal carbon, forming the six-membered alkyl palladium intermediate **II**. In our system, β-elimination of HPdOAc from **II** surpasses β-elimination of ArPdOAc to afford the intermediate **III** [12]. It is noticeable that no trace of methyl salicylate was detected when the crude product was analyzed by ^1H NMR. The ^1H NMR spectrum of the reaction intermediate shows the possibility that intermediate **III** may exist (See the Supporting Information-2). The addition–elimination of Pd(II) in this process is reversible. Since this reaction intermediate (**III**) is so stable that it can be isolated, the β-elimination of ArPdOAc apparently does not proceed. The 1,4-diene **III** is further oxidized by $Pd(OAc)_2$ through the intermediate **IV** to provide **V**. Enolization of ketone **V** leads to the thermodynamically stable aromatic compound **5**. HPdOAc releases AcOH and becomes Pd(0), which could be oxidized by $Cu(OAc)_2$ and an oxygen to generate $Pd(OAc)_2$.



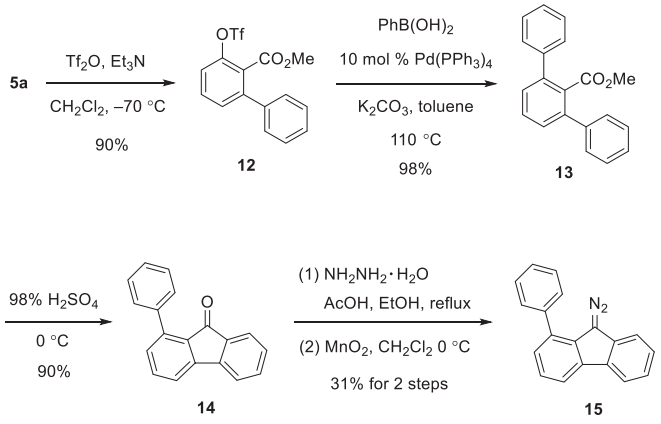
Scheme 4. One-pot synthesis of substituted biphenyls.



Scheme 5. Proposed reaction mechanism.



Scheme 6. Total synthesis of 9-diazo-1-methoxy-9*H*-fluorene (**4**).



Scheme 7. Total synthesis of 9-diazo-1-phenyl-9H-fluorene (**15**).

Since the biphenyl **5a** was obtained in a 60% yield, began the total synthesis of 1-methoxydiazofluorene **4** according to the proposed functional group transformations by SYNSUP. Methylation of the alcohol **5a** with methyl iodide in the presence of K_2CO_3 gave rise to **9** (95%), which was cyclized using 98% H_2SO_4 to furnish fluorenone **10** in an 84% yield [13]. Refluxing of **10** with hydrazine monohydrate in the presence of acetic acid provided the hydrazone **11** in a 94% yield [14], which led to 9-diazo-1-methoxy-9*H*-fluorene (**4**) in an 87% yield by MnO_2 oxidation [15]. The spectroscopic properties of synthetic material **4** were identical with those reported for **4** [8] (Scheme 6).

Because a series of conversion reactions from the biphenyl compound **5** seemed to be effective for a concise synthesis of various diazofluorenes, we decided to carry out antitumor activity tests of various novel diazofluorenes using human cancer cells. Compound **15,** having a phenyl group at the 1-position of compound **4,** was synthesized as follows (Scheme 7) (See Supporting Information-2).

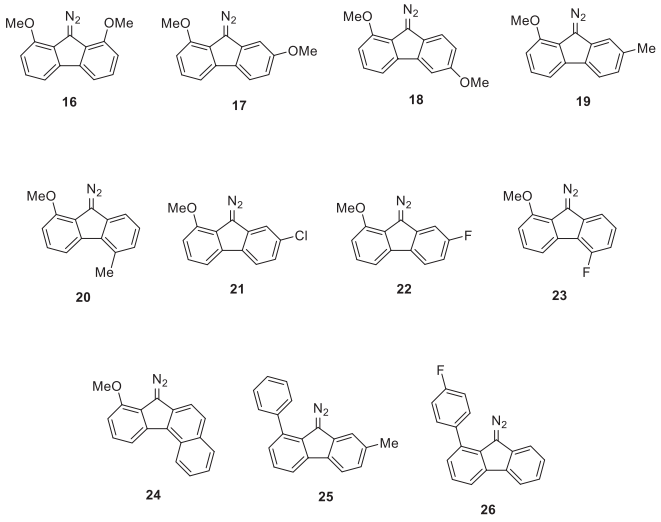


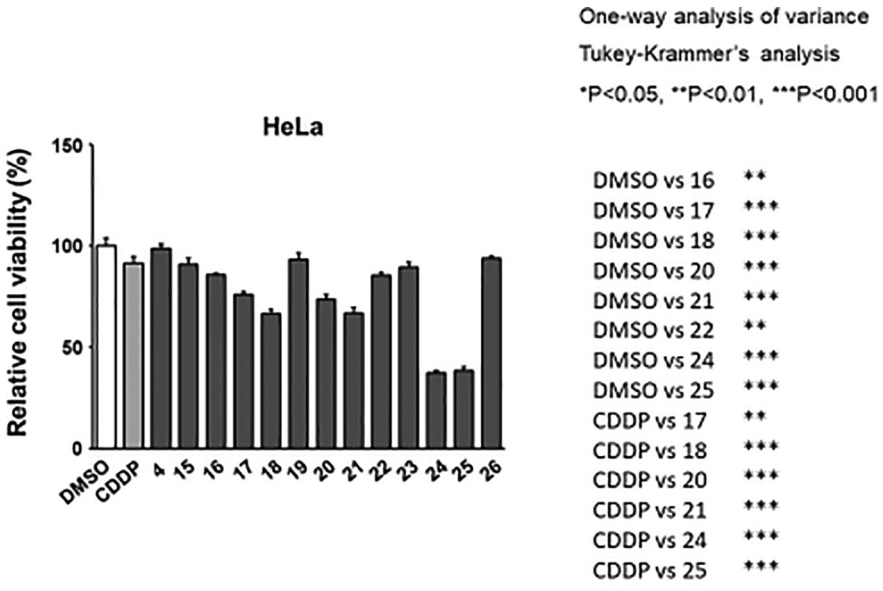
Fig. 1. Structures of novel synthetic diazofluorenes **16**–**26**.

After triflate formation of **5a**, a Suzuki-Miyaura coupling reaction [16] with phenylboronic acid afforded terphenyl **13** in an excellent yield. Intramolecular Friedel-Crafts reaction of **13** in the presence of 98% H_2SO_4 gave 1-phenyl-9H-fluoren-9-one (**14**), which was transformed into the desired 1-phenydiazo-fluorene **15** (Scheme 7).

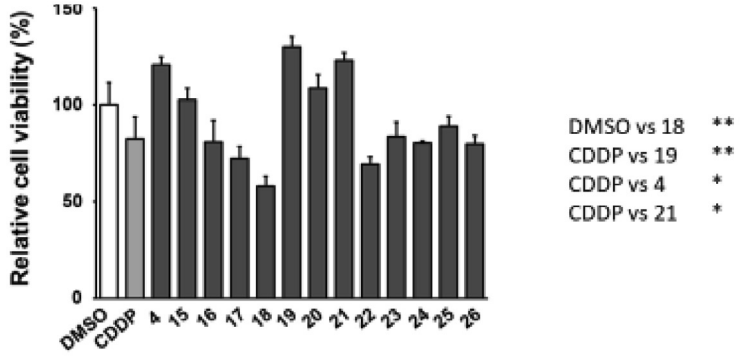
Diazofluorenes **16**–**24** were prepared from the corresponding biphenyls **5** using the same synthetic route as for **4**. On the other hand, 1-aryldiazofluorenes **25** and **26** were synthesized by applying the same approach as for **15** (Fig. 1). Each of the synthetic diazofluorenes were easily purified by flash column chromatography. It should be noted that most synthetic diazofluorenes including **4**, and **15**–**26** are stable enough not to decompose even if left in a nitrogen atmosphere at room temperature for about a week.

Cytotoxicity test

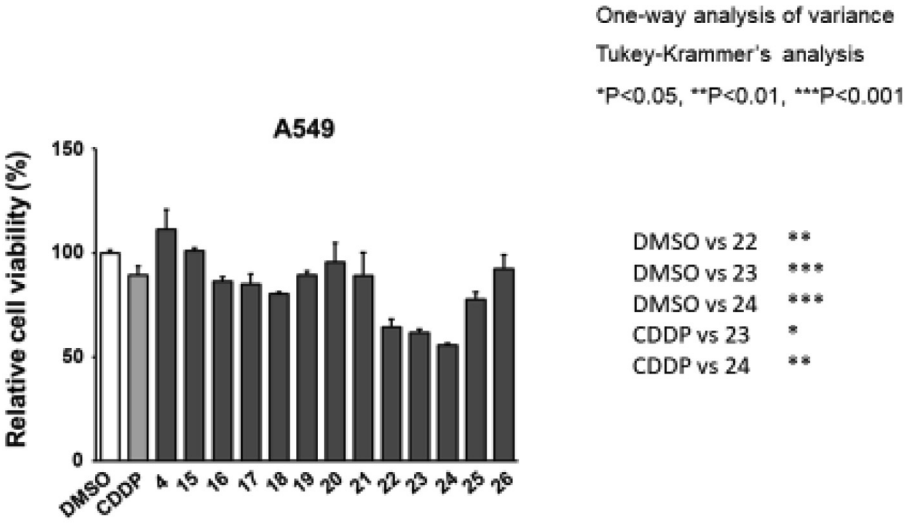
Since natural kinamycins are not commercially available, we selected cisplatin (CDDP) [17] as a standard to evaluate the strength of antitumor activity of the novel diazofluorenes. Cytotoxicity tests of synthetic diazofluorenes **4**, and **15**–**26** using HeLa, PK1, A549, BT474, and SW1116 human cancer cells were performed. Compared with CDDP, compounds **24** and **25** showed stronger cytotoxic activities against HeLa cervical cancer cells. Compound **18** was very effective against PK1 pancreatic cancer cells. Additionally, compounds **22**–**24** showed strong cytotoxic activities against A549 lung, BT474 breast, and SW1116 colon cancer cells [18]. Details with statistical analysis are described in Supporting Information-3.



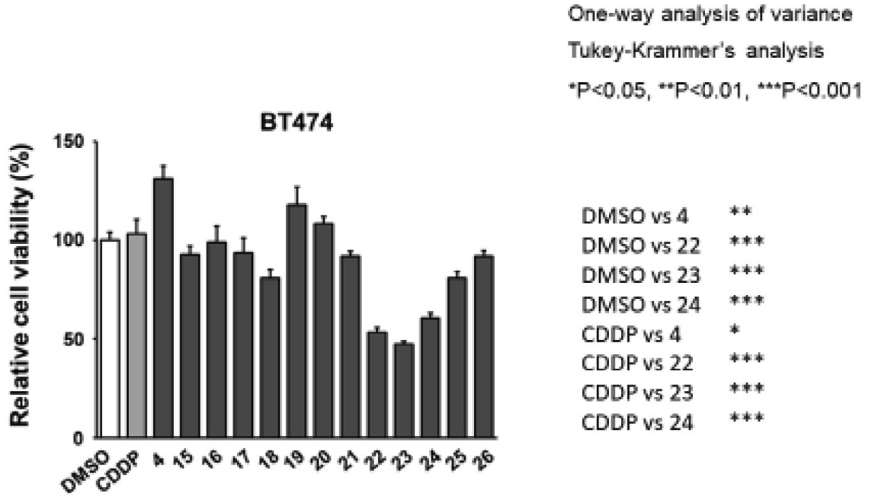
Effects of various diazofluorenes on the growth of Hela uterus cancer cells



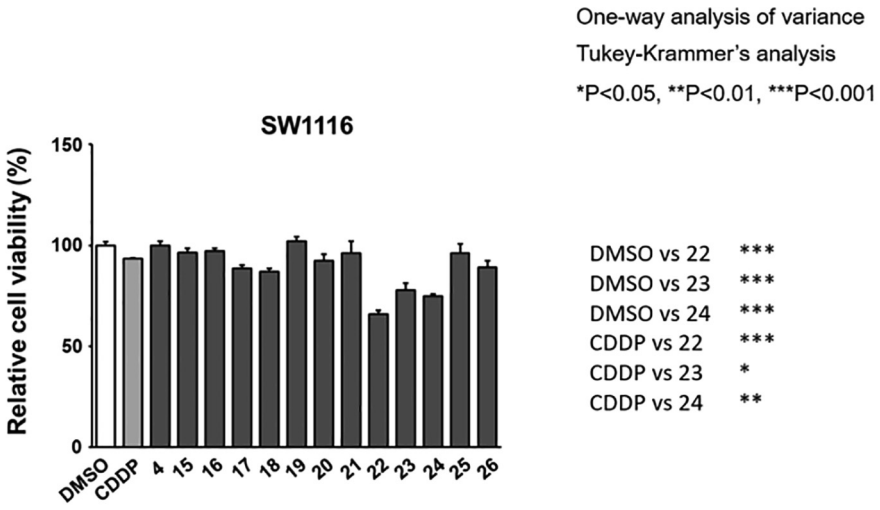
Effects of various diazofluorenes on the growth of PK-1 pancreatic cancer cells



Effects of various diazofluorenes on the growth of A549 lung cancer cells



Effects of various diazofluorenes on the growth of BT474 breast cancer cells



Effects of various diazofluorenes on the growth of SW1116 colon cancer cell

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We would like to thank Sumitomo Chemical for allowing us to use SYNSUP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152267.

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