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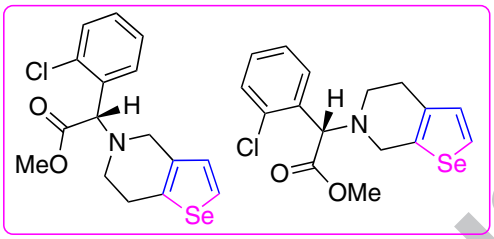

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Selenium analogues of (*S*)-clopidogrel: preparation method and properties

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ABSTRACT

A simple approach to prepare selenium containing analogues of (*S*)-clopidogrel using selenophene ring construction was developed. Evaluation of the antiproliferative activity profile confirmed that the introduction of a selenium atom into the molecule of clopidogrel simultaneously increased the antiproliferative activity against non-Hodgkin's lymphoma Mino cells and decreased the toxicity.

(*S*)-Clopidogrel or (+)-*S*-methyl-2-(2-chlorophenyl)-2-(6,7)-dihydrothieno[3,2-*c*]pyridin-5(*4H*)-yl)acetate is an oral drug marketed under the trade name Plavix.[1] Its action is based on decreasing platelet aggregation and inhibiting thrombus formation. This antiplatelet agent is often used to prevent myocardial infarction in the treatment of coronary artery disease, peripheral vascular disease and cerebrovascular disease. Preparative methods for [3,2-*c*]-annulated thienopyridine derivatives and the properties of these compounds have been well described in reviews.[2] Furthermore, in the last decades, selenium has attracted growing interest as an essential element for human health. Supplemental dietary selenium is associated with the reduced incidence of many types of cancers.[3] However, the harmful effects of selenium overdose on human health should also be considered. The recommended dietary allowance of selenium for adults is up to 55 μg per day.[4] In a continuation of our studies in the field of selenium-containing compounds[5], we herein report the synthesis of selenium analogues of clopidogrel and compare their antiproliferative activity on various tumour cell lines with that of clopidogrel.

A wide range of studies have been devoted to the synthesis of selenophene condensed with *N*-heteroaromatic rings.[6] Meanwhile, many chalcogen-containing compounds with practical value remain difficult to access. Thus, the creation of new and convenient preparative methods is a fundamental problem in synthetic chemistry. Previously we have reported selenophene ring formation on aryl and heteroaryl substrates and the insertion of a selenophene fragment on non-aromatic coumarin and quinolinone rings.[7] These synthetic methods were based on the reaction of ethynyl derivative with selenium halides.

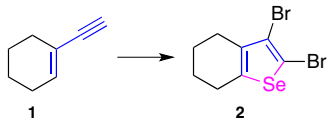
In the present work, given the non-aromatic character of the clopidogrel dihydrothienopiridinyl moiety, we selected commercially available 1-ethynylcyclohexene (**1**) as a model compound to optimize the reaction conditions (Table 1). Our study began with the reaction of **1** with 2 equiv. of *in situ* prepared $SeBr_2$ which was prepared from Se powder and Br_2 in CCl_4 in the dark to avoid disproportionation of the reagent (Entry 1). Substrate **1** was added to $SeBr_2$ at 0 °C, and the reaction mixture stirred at room temperature (20 °C). In accordance with GC-MS data, after 24 h of stirring, a mixture of polybrominated compounds with high molecular weights was formed.

Next, we utilised commercially available $SeBr_4$ and an equimolar amount of cyclohexene reagent was used as a bromine molecule scavenger since during cyclization a bromine molecule can add to the triple and double bonds of the starting material and dramatically decrease the product yield (Entry 2). The reaction was performed at room temperature, and subsequent GC-MS analysis indicated that the product was a mixture of several products: dibromocyclohexane, 1,2-dibromo-1-ethynylcyclohexane, compounds with high molecular weights, and only 11% of the desired 2,3-dibromo-4,5,6,7-tetrahydrobenzo[*b*]selenophene (**2**).

Disappointed with the outcome of previous attempts, we further optimized the reaction conditions using $SeBr_4$ which was prepared *in situ* by dissolving SeO_2 in concentrated hydrobromic acid. This source of selenium halide enabled us to decrease the cost of the reagents. In order to minimize the probability of the formation of compounds with high molecular weights compound **1** was added to $SeBr_4$ at reduced temperature and the amount of the bromine scavenger was increased to a ratio of 1:2 (Entry 3). In this case, two major products were obtained: 3-bromo-4,5,6,7-tetrahydrobenzo[*b*]selenophene (10%) and 2,3-dibromo-4,5,6,7-tetrahydrobenzo[*b*]selenophene (**2**, 32%) (Entry 3). To obtain a single major product, we then varied the amount of scavenger. Thus, when equimolar amounts of SeO_2 and cyclohexene were used (Entry 4), the yield of product **2** increased to 35%, whereas

bromination of the initial compound was not observed. A reduction of the amount of cyclohexene to 0.5 equiv. increased the yield of product **2** to 49%. Finally, the reaction without scavenger was examined (Entry 6). The simplest reaction conditions appeared to be preferable because of the high reactivity of the triple bond in **1**. After workup and purification, pure 2,3-dibromo-4,5,6,7-tetrahydrobenzo[*b*]selenophene (**2**) was isolated in 64% yield.

Table 1. Ethynylcyclohexene reaction with selenium bromides



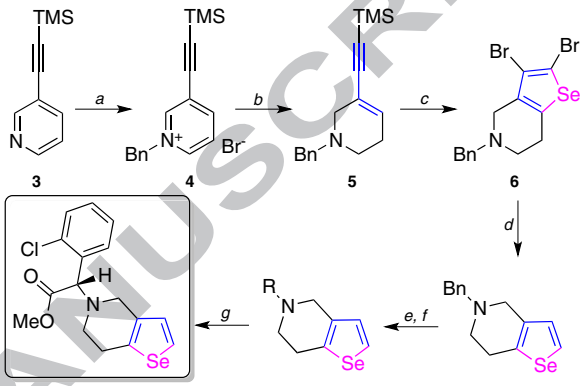
1 2

Entry	$SeBr_n$	Cyclohexene	Yield (%)
1	$SeBr_2$ (2 equiv.)	-	0
2	$SeBr_4$ (1.3 equiv.)	1.3 equiv	11
3	SeO_2/HBr (1 equiv.)	2 equiv	32
4	SeO_2/HBr (1 equiv.)	1.0 equiv	35
5	SeO_2/HBr (1 equiv.)	0.5 equiv	49
6	SeO_2/HBr (1 equiv.)	-	64

Given the aforementioned results, synthesis of the corresponding ethynyltetrahydropyridine was required to enable the subsequent preparation of the desired tetrahydroselenophenopyridine derivative. Because pyridinium salts are widely used to synthesize cyclic tetrahydropyridines, compound **4** was prepared by quaternizing ethynylpyridine **3** with benzyl bromide in dichloromethane (Scheme 1). After solvent evaporation, the precipitate was washed with diethyl ether and pure *N*-benzyltetrahydropyridine derivative **4** was isolated in 60% yield by filtration. Then, excess sodium borohydride was carefully added to a solution of 1-benzyl-3-((trimethylsilyl)ethynyl)pyridinium bromide (**4**) in methanol at -10 °C, and the reaction mixture stirred overnight at room temperature. After the evaporation of methanol and workup, derivative **5** was isolated in 63% yield using flash column chromatography.

The cyclisation reaction of compound **5** with *in situ* prepared $SeBr_4$ was performed using the procedure previously described for ethynylcyclohexene. Notably, after 24 h, the reaction mixture contained 5-benzyl-2,3-dibromo-4,5,6,7-tetrahydroselenopheno[3,2-*c*]pyridine (**6**) and approximately 30% of the 5-benzyl-3-bromo-2-trimethylsilyl-4,5,6,7-tetrahydroselenopheno[3,2-*c*]pyridine derivative. Complete desilylation and conversion to dibromo product **6** was achieved after an additional portion of *in situ* prepared $SeBr_4$ (0.5 equiv.) was added. Hence, 5-benzyl-2,3-dibromo-4,5,6,7-tetrahydroselenopheno[3,2-*c*]pyridine (**6**) was isolated in 50% yield.

One of the most common methods used to synthesize (*S*)-clopidogrel is 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine alkylation using (*R*)-4-nitrobenzenesulfonyloxy-2-(2-chlorophenyl)acetate.[8a] Thus, synthesis of the clopidogrel selenium analogue required debromination and *N*-deprotection of compound **6**. Attempts to obtain the desired compound **9** in a single step using the Pd/C-catalysed reduction were unsuccessful and only resulted in debromination, giving 5-benzyl-4,5,6,7-tetrahydroselenopheno[3,2-*c*]pyridine (**7**) in 83% yield. Remarkably, when a classical method to remove bromo substituents (reflux, 80% aqueous acetic acid, Zn powder) was used, the reaction time was substantially increased and the overall yield of **7** was only 20%. For cleavage of the benzyl group, a two-step procedure was used. First, *N*-acylation with methyl chloroformate proceeded in 73% yield which was followed by heating 6,7-dihydroselenopheno[3,2-*c*]pyridine derivative **8** at reflux in a mixture of methanol and 2 M NaOH (aq.) for 24 h resulting in cleavage of the methyl carboxylate in 98% yield. Finally, alkylation of the free base **9** with excess (*R*)-4-nitrobenzenesulfonyloxy-2-(2-chlorophenyl)acetate in DCM at reflux gave the desired (*S*)-clopidogrel selenium analogue **10** in 81% isolated yield. (*R*)-4-Nitrobenzenesulfonyloxy-2-(2-chlorophenyl)acetate was prepared from (*R*)-*o*-chloromandelic acid.[8b,c]

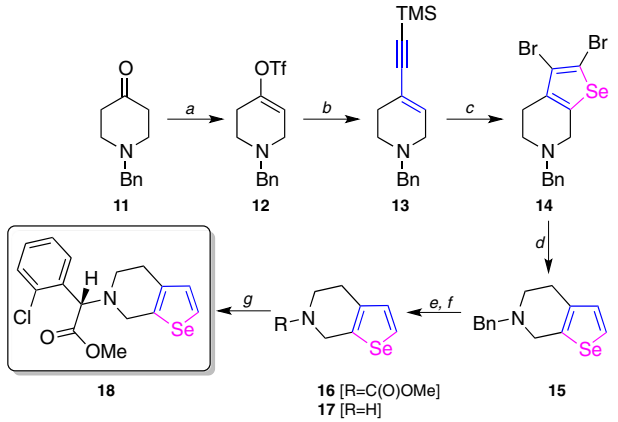


Scheme 1. Synthetic procedure to prepare **10**. *Reaction conditions*: (a): BnBr (1.5 equiv.), CH_2Cl_2, r.t., 60%; (b): $NaBH_4$ (3.5 equiv.), MeOH, -10 °C to r.t., 63%; (c): SeO_2/HBr (1.2 equiv.), dioxane, 0 °C to r.t., 50%; (d): 10% Pd/C (0.5 equiv.), H_2, EtOH/THF/HCOOH = 5/1/0.1, r.t., 83%; (e): MeOC(O)Cl (7 equiv.), 0 °C to r.t., 73%; (f): 2 M NaOH, MeOH, reflux, 98%; (g): (*R*)-4-nitrobenzenesulfonyloxy-2-(2-chlorophenyl)acetate (1.7 equiv.), 30% aqueous solution of K_2CO_3, CH_2Cl_2, reflux, 81%.

The method developed to synthesize selenopheno[3,2-*c*]tetrahydropyridine **6** was also used to obtain its analogue **14** (Scheme 2). The required starting material **13** was synthesized from commercially available *N*-benzylpiperid-4-one (**11**). LHMDS was added to a solution of **11** at -78 °C, and, after 30 min, the flask was removed from the cold bath for 10 min. A solution of *N*-phenyl-bis(trifluoromethanesulfonimide) in THF was then added dropwise at -78 °C, and the mixture stirred overnight to warm to rt. After workup, compound **12** was isolated in 82% yield. Attempts using *n*-BuLi or LDA instead of LHMDS led to undesired results. In the next step, the Sonogashira protocol, mediated by $Pd(PPh_3)_4$/CuI in DMF/DIPA at rt, was used to give 1-benzyl-4-((trimethylsilyl)ethynyl)-1,2,3,6-tetrahydropyridine (**13**) in 65% yield. The cyclization reaction of **13** was carried out using 1.2 equiv. of *in situ* prepared $SeBr_4$, stirring at 0 °C for 48 h to give tetrahydroselenopheno[2,3-*c*]pyridine **14** in 44% yield. Finally, compound **18** was obtained using procedures similar to those previously described to obtain (*S*)-clopidogrel selenium analogue **10**.

Because both thienopyridines and selenium-containing compounds can be useful as antiproliferative substances, we continued our studies to improve the effects of introducing selenium atoms in drug design. The *in vitro* antiproliferative activity of clopidogrel and its selenium analogues was tested on monolayer tumour cell lines HL-60 (Human promyelocytic leukaemia), Jurkat (human T lymphocyte), U937 (histiocytic lymphoma), Mino (human peripheral blood/Mantle cell

lymphoma (B cell non-Hodgkin's lymphoma)), Raji (human Burkitt's lymphoma), DG-75 (metastatic Burkitt's lymphoma), and HepG2 (hepatocellular carcinoma). Using the NIH 3T3 (mouse fibroblast) cell line, we determined the borderline concentration, which is relevant to the highest tolerated dose, for each compound. The basal cytotoxicity is expected to be used to predict the starting doses for *in vivo* acute oral LD_{50} values in rodents (Table 2).



Scheme 2. Synthetic procedure used to prepare **18**. *Reaction conditions*: (a): 0.8 M LHMDS (1.2 equiv.), $PhN(Tf)_2$ (1.2 equiv.), THF, -78 °C to -20 °C, -78 °C to r.t., 82%; (b): trimethylsilylacetylene (1.5 equiv.), 10% $Pd(PPh_3)_4$, 12% CuI, DMF/DIPEA, r.t., 65%; (c): SeO_2/HBr (1.2 equiv.), dioxane, 0 °C to r.t., 44%; (d):10% Pd/C (5 equiv.), H_2, EtOH/THF/HCOOH = 5/1/0.1, r.t., 72%; (e): MeOC(O)Cl (7 equiv.), 0 °C to r.t., 83%; (f): 2 M NaOH, MeOH, reflux, 95%; (g): (*R*)-4-nitrobenzenesulfonyloxy-2-(2-chlorophenyl)acetate (1.7 equiv.), 30% aq. solution of K_2CO_3, CH_2Cl_2, reflux (83%).

Table 2. In vitro cytotoxicity (IC_{50}, μM) in monolayer tumour cell lines and normal cell line NIH3T3 (mouse fibroblasts).

Cell line	Clopidogrel	7	10	15	18
HL-60	81±7	102±26	81±19	153±22	71±22
Jurkat	33±7	43±12	27±10	56±6	26±8
U937	45±9	86±15	60±10	32±12	54±11
Mino	76±18	134±32	24±8	125±9	43±18
Raji	88±9	131±51	81±11	291±41	114±32
DG-75	88±19	195±19	144±16	70±22	92±16
HepG2	74±14	61±12	54±13	224±16	79±8
LD_{50}, (mg/kg)	546	1313	848	1438	811

By inspecting the cytotoxic profile of clopidogrel, we concluded that this drug exhibited a medium cytotoxic effect on the studied tumour cell lines (IC_{50} varied from 33 to 88 μM). Jurkat and histiocytic lymphoma U937 cells were more sensitive to clopidogrel and the basal toxicity was detected at LD_{50} = 546 mg/kg. Surprisingly, its selenium analogue **10** exhibited an extended cell type selectivity. Compared to clopidogrel, derivative **10** exhibits a good antiproliferative effect against non-Hodgkin's lymphoma Mino cells (IC_{50} = 24 μM). Moreover, the basal toxicity data confirmed that **10** exhibited a substantially lower toxicity than clopidogrel. In a similar manner to derivative **10**, selenium isoster **18** showed better cytotoxicity using the Mino cell line (IC_{50} = 43 μM) than clopidogrel (IC_{50} = 76 μM) and a moderate projected LD_{50} value of 811 mg/kg. Notably, the simple *N*-benzyl analogue **15** exhibited a slight better effect on U937 than clopidogrel.

In summary, we have developed a simple approach to construct the selenophene ring on a non-aromatic cyclohexene molecule. A new simple synthetic procedure was used to prepare selenopheno[2,3-*c*] and [3,2-*c*]dihydropyridines. As a result, a convenient protocol to synthesize the (*S*)-clopidogrel selenium analogue **10** and its isomer **18** was developed. The introduction of a selenium atom into the clopidogrel molecule increased its antiproliferative activity toward non-Hodgkin's lymphoma Mino cells. The obtained data results open a route to further modifications of a series of analogues of selenopheno[2,3-*c*]- and [3,2-*c*]dihydropyridines which may lead to a compound with extended activity against tumours.

Supporting Information

Supporting information contains full experimental data, copies of the ^{1}H and ^{13}C NMR spectra of all compounds.

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