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Synthesis and CHK1 inhibitory potency of Hymenialdisine analogues

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ABSTRACT

A series of thieno[3,2-*b*]pyrroloazepinones derivatives related to Hymenialdisine were prepared and tested for CHK1 inhibitory activity. Nanomolar inhibitions were achieved when electron-withdrawing substituents were introduced at position 3 of the thiophene ring.

Over the last decades, DNA damaging agents have been used as first line agents in cancer chemotherapy. As a consequence of DNA damage by UV light, radiation, or cytotoxic drugs, tumor cells arrest their cycle in S or G_2/M phases to attempt DNA repair or undergo apoptosis.[1] Studies have demonstrated that the Serine/Threonine checkpoint kinase CHK 1 regulates both the G_2/M and intra-S checkpoints and plays an important role in cell-cycle progression, mainly for the P53-defective cancer cells (50–70% of all cancers). Since cell-cycle arrest was found to be necessary for DNA repair following damages induced by genotoxic agents, abrogation of the G_2/M checkpoint by specific inhibitors should increase the sensitivity of P53 deficient tumours to DNA damaging agents without enhancing toxicity toward normal proliferating cells, which are non-mutated P53 proficient cells.[2,3]

(*Z*)-Hymenialdisine **1**, (*Z*)-2-debromohymenialdisine **2** and (*Z*)-3-bromohymenialdisine **3** (Fig. 1), are three naturally occurring alkaloids isolated from the marine sponges Hymenialcidon, Acanthella, Axinella and Stylissa.[4] Structurally these compounds bear a pyrrole ring, often substituted with one or two bromine atoms, as well as an imidazolinone. These compounds have been reported to inhibit a large panel of kinases, among which CHK1.[5,6]

In 2001, Smith-Kline–Beecham patented a series of Myt1 inhibitors exemplified here by the indole derivative **4**.[7] This compound was later described by Tepe as a potent CHK1 inhibitor and its preparation was given.[8] In our hands, in vitro potency and selectivity as well as in vivo activity of this family of inhibitors showed that improvement was needed in order to obtain a candidate for development. X-ray crystallography of Debromohymenialdisine in the ATP binding site of apo human CHK1 suggested that structural opportunities may still exist to enhance affinity against the enzyme by filling a pocket in the pyrroloazepinone region.[9] Thus, we decided to explore the replacement of the phenyl ring of the indole system in **4** with other heterocycles. We report here our efforts in preparing and evaluating the potency against CHK1 of thieno-[3,2-*b*]-pyrroloazepinones analogues of *Z*-hymenialdisine **1**.

The synthesis of this family of compounds is depicted in Scheme 1. Conveniently substituted 2-formylthiophenes **5** were engaged in an alkylidenation reaction with ethyl 2-azido acetate in the presence of sodium ethoxide as a base according to the strategy of Hemetsberger and Knittel.[10] Cyclisation of the ethyl azido thiophene carboxylates **6** was realized in refluxing xylene and gave the ethyl thieno[3,2-*b*]pyrrole-5-carboxylates **7**. The corresponding acids **8** were obtained by saponification in a mixture of aqueous sodium hydroxide and ethanol in quantitative yields. Coupling these acids with β-alanine ethyl ester gave esters **9** in good yields. A second saponification using aqueous potassium hydroxide in refluxing ethanol allowed us to obtain the acids **10** in very good yields. Cyclisation of these compounds to give the thieno[3,2-*b*]pyrroloazepinones **11** proved to be more tedious: the expected compounds were only obtained using a mixture of $MeSO_3H$ and P_2O_5 warmed at 80–100 °C for 1–4 h with yields ranging from 15% to 93%. Protection of the nitrogen atom of the pyrrole ring was realized with *p*-methoxybenzyl bromide to give compounds **12**. At this stage, this nitrogen atom was also methylated (K_2CO_3, CH_3I, DMF, 63%). To incorporate the glycocyamidine ring, we capitalized on the observation made by Tepe et al.,[11] and succeeded in condensing thiohydantoine and rhodanine with ketones **12** using the previously

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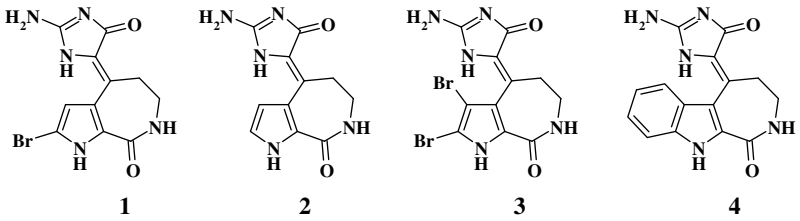


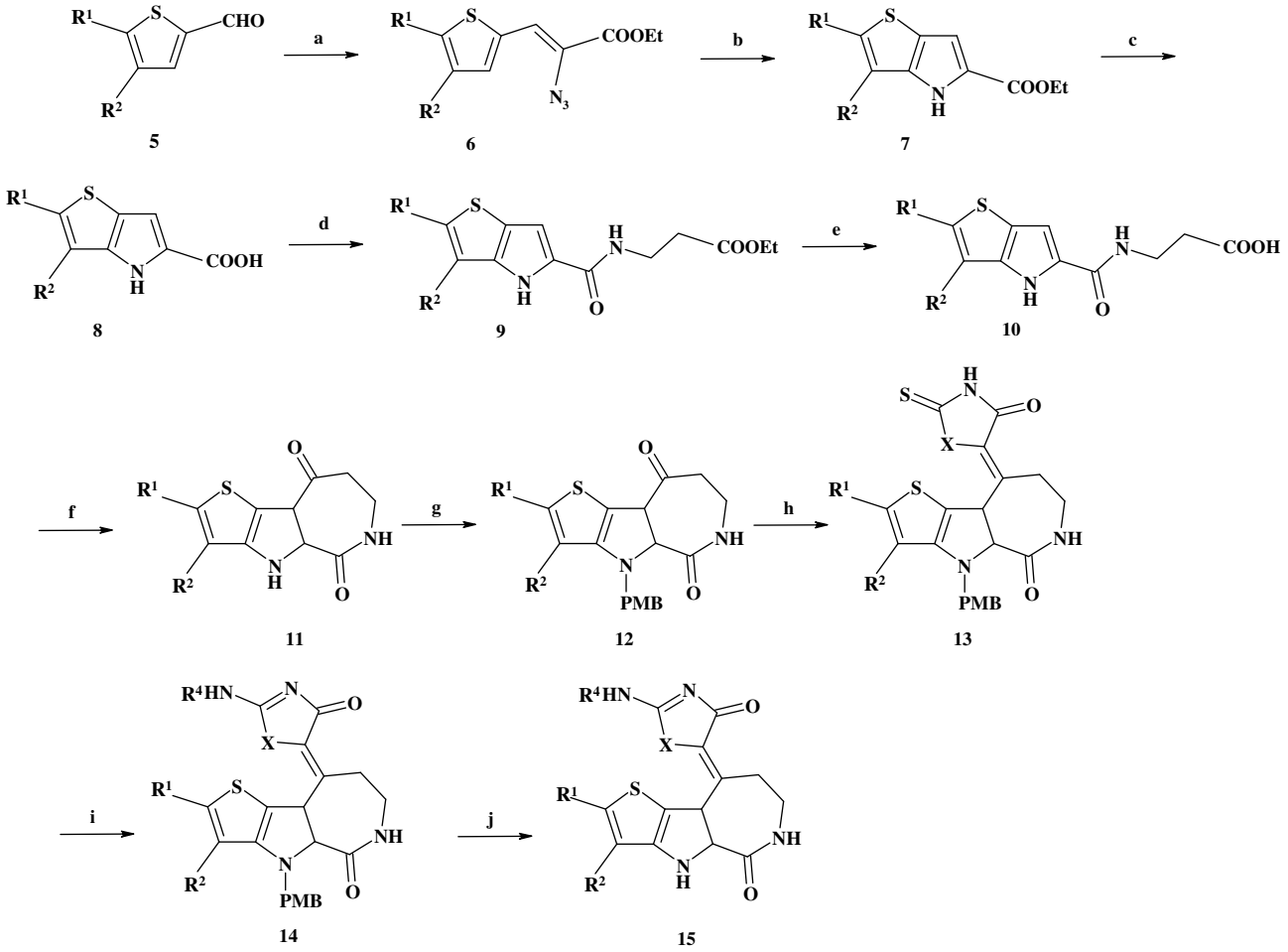
Figure 1.

reported Ti(IV)-pyridine reagent. Yields of **13** were predominantly low to moderate. These intermediates were further transformed into Hymenialdisine analogues by reacting **13** with a variety of amines in the presence of terbutyl-hydroperoxide as an oxidant,[12] to produce **14**. **14p** and **14q** were obtained by condensation with ethanolamine and **14r** with morpholin-1-yl-ethylamine.

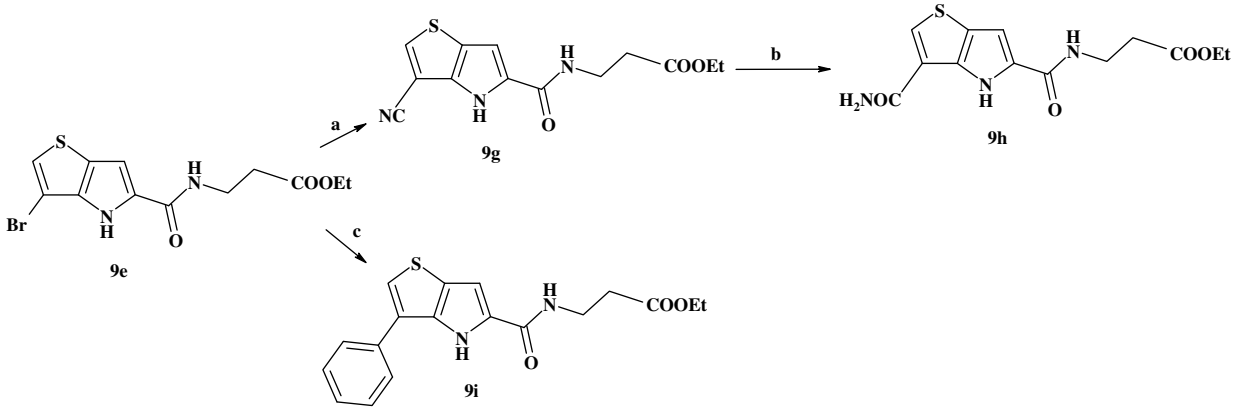
Otherwise, ammonia was used. Final deprotection of the thieno[3,2-*b*]pyrroloazepine moiety with trifluoroacetic acid afforded the final inhibitors **15** after reverse phase HPLC purification. The preparation of **15o** necessitated two more steps, first coupling with Boc-glycine (HATU, DIEA, DMF, 48 h, rt, 62%) followed by deprotection with TFA (CH_2Cl_2, 2 h, rt, 42%).

Compound **9e** was found to be the starting material for structural modifications at position 3 on the thieno[3,2-*b*] pyrrole ring (Scheme 2). Substitution of the bromide was realized by standard cyanation conditions resulting in the obtention of **9g**, which was subsequently hydrolysed in acidic media to give **9h**. On the other hand, introduction of a phenyl group at position 3 of **9e** was successfully realized by a Suzuki–Miyaura reaction involving phenyl boronic acid according to the conditions described by Buchwald to produce **9i** in excellent yield.[13] These intermediates were engaged in the following steps as described above.

IC_{50} for Chk1 inhibition are given in Table 1. First, it is worth noting that the potencies of the debromo and dibromo derivatives **2** and **3**, as well as the indole analog **4** were found in the same range of activity at IC_{50} between 0.10 and 0.47 μM, indicating that substitutions on the pyrrole ring as well as its coupling with a phenyl to give the indole ring hardly influenced activity. Hymenialdi-

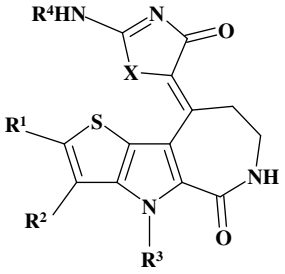


Scheme 1. Typical synthesis of inhibitors. Reagents and conditions: (a) ethyl 2-azido acetate, EtONa, 2 h, 20–55%; (b) xylene, reflux, 0.5 h, 70–75%; (c) 1 N NaOH, EtOH–H_2O, 20 h, 52–95%; (d) β-alanine ethyl ester, DCC-HOBT-DIEA, DMF, 2 h, 80–100%; (e) 1 N KOH, EtOH–H_2O, 3–6 h, 85–97%; (f) CH_3SO_3H–P_2O_5, 1–4 h, 15–93%; (g) *p*-methoxybenzyl bromide (PMB-Br), K_2CO_3, CH_3CN, 4–10 h, 75–85%; (h) thiohydantoine (X = NH) or rhodanine (X = S), TiCl4-pyridine-THF, 3 h at −5 °C, 11–62%; (i) R4-NH_2, *t*BuOOH–EtOH, 8 h, 12–55%; (j) refluxing TFA, 12 h, 25–52%.



Scheme 2. Modifications of **9e** at position 3. Reagents and conditions : (a) $Cu(CN)_2$, DMF, 6 h, 41%; (b) 4 N HCl, dioxane, 100 °C, 3 h, 64%; (c) phenyl boronic acid, $Pd_2(dba)_3$, 2′-dicyclohexylphosphino-2,6-dimethoxy-1,1′-biphenyl (S-Phos), K_3PO_4, toluene, 10 h, 64%.

Table 1
ChK1 IC_{50} values for compounds **1**–**4**, **14a**–**b**, **15a**–**s**



Compound	R^1	R^2	R^3	R^4	X	CHK1 IC_{50} (μM)
1	—	—	H	H	NH	1.9*
2	—	—	H	H	NH	0.33
3	—	—	H	H	NH	0.10
4	—	—	H	H	NH	0.47
14a	H	H	CH_3	H	NH	>10
14b	H	Br	4-MeOBn	H	NH	>10
15a	H	H	H	H	NH	0.15
15b	Cl	H	H	H	NH	0.30
15c	H	Cl	H	H	NH	0.14
15d	Cl	Cl	H	H	NH	0.89
15e	Br	H	H	H	NH	0.46
15f	H	Br	H	H	NH	0.014
15g	H	CN	H	H	NH	0.029
15h	H	$CONH_2$	H	H	NH	0.15
15i	H	Ph	H	H	NH	1.3
15j	Phenyl ring		H	H	NH	0.23
15k	H	H	H	H	S	0.18
15l	H	Br	H	H	S	0.19
15m	H	Ph	H	H	S	>10
15n	Phenyl ring		H	H	S	>10
15o	H	Br	H	$HOOC-CH_2$	NH	0.26
15q	H	H	H	$HO(CH_2)_2$	NH	1.7
15r	H	Br	H	$HO(CH_2)_2$	NH	0.28
15s	H	H	H	Morpholin-1-ethyl	NH	>5

* Literature value Ref. 8.

sine **1** was not tested in house, but its IC_{50} value reported by Tepe at 1.9 μM can be considered if one compares IC_{50}'s for DBH **2** at 0.72 μM in Tepe's paper and 0.33 μM in our hands. As far as the thieno-[3,2-*b*]-pyrroloazepinone ring system was concerned, we studied first the alkylation of the nitrogen atom of the central pyrrole ring. A methyl group in **14a** or a *p*-methoxy benzyl group in **14b** suppressed the activity, with IC_{50} greater than 10 μM. This set us to thinking that this nitrogen atom could be engaged in a donor–acceptor bond with the active site of the enzyme. Suppressing this substitution in **15a** restored an inhibitory activity similar to that of the reference molecules, with an IC_{50} of 0.15 μM. Equivalent activities were found when a chlorine or a bromine atom were introduced at position 2 (**15b**: 0.3 μM and **15e**: 0.46 μM) or when a chlorine atom was introduced at position 3 (**15c**: 0.14 μM). The 2,3-dichloro substitution present in compound **15d** slightly decreased the activity with an IC_{50} of 0.89 μM. The breakthrough in these structural studies was obtained when bromine or nitrile were introduced at position 3, giving rise to a substantial gain in

activity for inhibitors **15f** and **15g** with IC_{50} of 0.014 and 0.029 μM, respectively. Conversion of the nitrile moiety to an amide in **15h** resulted in loss of potency at 0.15 μM. Introduction of a phenyl ring at position 3 in **15i** was rather deleterious for activity with an IC_{50} of 1.3 μM, but fusing this phenyl with the thiophene ring in compound **15j** restored the activity, giving an IC_{50} of 0.23 μM.

We then turned our attention to the imidazolinone ring. The protonated nitrogen atom was replaced by a sulphur atom to give the more lipophilic rhodanine derivative **15k** which maintained the inhibitory potency at 0.18 μM. Curiously enough, one order of magnitude in the potency was lost with the 3-bromo derivative **15l** (0.19 μM vs 0.014 μM for **15f**). Furthermore, **15m** and **15n**, the analogs of compounds **15i** and **15j**, were found to be barely active, with activities greater than 10 μM.

Finally, substitution of the exocyclic nitrogen atom of the imidazolinone ring gave compounds **15o–s**. Activity was decreased of one order of magnitude compared to the non-substituted parent compounds **15a** or **15f**.

In summary, we explored a new series of thieno-[3,2-*b*]-pyrroloazepinones analogues of the natural Hymenialdisine family. Substitution of the thiophene ring with electron-withdrawing groups such as bromo or cyano afforded novel inhibitors **15f** and **15g** which produced a 10-fold improvement in the enzymatic inhibitory potency at IC_{50} of 14 and 29 nM against Chk1, respectively. In contrast, small structural variations located on the imidazolinone ring resulted in extensive erosion of inhibitory activity. **15f** was submitted to a selectivity profiling against a panel of 220 kinases. Like Hymenialdisine and several other CHK1 inhibitors, **15f** proved to be a multi-targeted kinase inhibitor, giving 52 hits (>80% inhibition at 1 μM). Nanomolar IC_{50} were found for the inhibition of kinases such as FLT3, GSK3β, ARK5 or SIK. The lead compounds have also been evaluated at the cellular level as well as in vivo against a panel of tumoral cell lines. These results will be reported shortly.

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