RSC Medicinal Chemistry

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View Article Online
DOI: 10.1039/D6MD00278A

ARTICLE

Synthesis, molecular modelling and evaluation of (–)-isopulegol-based 2,4-diaminopyrimidines as promising Aurora kinase inhibitors

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A library of (–)-isopulegol based 2,4-diaminopyrimidines was prepared from commercially available (–)-isopulegol. Aminodiols, derived from (–)-isopulegol according to literature methods, were added to 5-substituted 2,4-dichloropyrimidines, then the resulting products were later applied in microwave-assisted S_NAr coupling reactions with aniline derivatives to produce 2,4-diaminopyrimidines. All 2,4-diaminopyrimidine adducts were evaluated for their *in vitro* cytotoxicity against human colon adenocarcinoma cell lines, including Colo205 and Colo320. Among these derivatives, compounds **6a** and **7b** exhibited significantly greater efficacy against two cancer cell lines within concentrations around 2.0 µM. Furthermore, these derivatives displayed higher selectivity of cancer cells over normal cells (SI > 44) compared to the positive controls, doxorubicin (SI > 2) and cisplatin (SI = 5). Molecular docking analysis indicated that these compounds (**6a** and **7b**) form an interaction with Aurora A kinase receptor both from the perspective of structure and energy. These results suggest that derivatives **6a** and **7b** have potential for further development as Aurora A kinase inhibitors for colorectal cancer treatment.

Introduction

The Aurora kinase family, including three kinases designated as Aurora A, B, and C is a subfamily of serine/threonine kinases that have emerged as crucial factors of, not only mitosis and cytokinesis, but also human carcinogenesis.[1,2] Among them, Aurora kinase A and B have received the most attention to date as anticancer targets.[3] Aurora A kinase, which plays a pivotal role in various cellular processes, including mitotic entry, centrosome maturation and spindle formation, is frequently overexpressed in tumour cells[4–7] while Aurora B kinase, which plays a role in cell cycle progression, is frequently linked to tumour cell invasion, metastasis and drug resistance.[8–10] Both Aurora kinase A and B have been validated as targets for anticancer drugs.[11–25]

2,4-Diaminopyrimidines, an important pharmacology core, exhibited potent inhibitory activity in a number of protein kinases (Figure 1)[26–28] including Aurora kinase PF-03814735,[29,30] Polo-like kinase 1 (PLK1) BI2536 and DAP-81,[31] Focal adhesion kinase (FAK) such as VS-6062, [32,33] CEP-37440[34] and NVP-TAE 226,[35,36] Spleen tyrosine kinase (SYK) R406,[37] Rad3-related kinase (ATR) NU6027,[38] Cyclin-dependent kinases (CDK) R547,[39] Multi-targeted tyrosine kinase XL228,[40] Anaplastic lymphoma kinase (ALK) such as NVP-TAE684[41] and GSK1838705A.[42] Some of them were approved by the FDA for cancer treatment including multi-targeted inhibitor Pazopanib (Votrient®, approved 2009),[43] ALK inhibitors such as Ceritinib (Zykadia®, approved 2014),[44] Brigatinib (Alunbrig®, approved 2017), [45] SYK inhibitor Fostamatinib (Tavalisse®, approved 2018),[46] JAK-2 inhibitor Fedratinib (Inrebic®, approved in 2019)[47] or in clinical trials such as SYK inhibitor Cerdulatinib (Phase 2 study),[48] PLK1 inhibitor Volasertib (Phase 2 study),[49] FAK inhibitor *Defactinib* (Phase 1 study).[50] The discovery of novel and selective inhibitors to protein kinases including Aurora A kinase,[51–58] Anaplastic lymphoma kinase (ALK),[59–66] Cyclin-dependent kinase (CDK2),[67,68] (CDK7),[69] (CDK9)[70,71] EGFR-targeted tyrosine kinase (EGFR-TK),[72–75] Focal adhesion kinase (FAK),[76–79] Tropomyosin receptor kinase (TRK),[80] p21-activated kinases (PAKs),[81] Janus kinase (JAK2),[82] (JAK3),[83] Glyoxalase I (GLO-1),[84] Mesenchymal epithelial transition factor (c-Met) kinase,[85,86] Hematopoietic Progenitor Kinase 1 (HPK1),[87–90] Casein kinase 1 epsilon (CK1ε)[91] continue to attract attention by many research groups.

Our previous work indicated that N^2-(*p*-trifluoromethyl)aniline substituted pyrimidines, prepared from (–)-isopulegol based aminodiols, displayed potent inhibitory effects on the growth of cancer cells.[92] Docking studies suggested that the pyrimidine core as well as the amine group of aminodiol ring form hydrogen bonds with the hinge region of the Aurora A kinase domain.[92] According to the literature, the displacement of the *p*-trifluoromethyl group on the aniline ring by substituents containing hydrophilic and acid-base interactions, such as carboxylic acid, carboxylic ester, and amide groups may lead to significant Aurora A kinase inhibitors.[55] Therefore,

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View Article Online DOI: 10.1039/D6MD00278A

in order to identify novel and highly potent Aurora A kinase inhibitors using the pyrimidine moiety, a new series of 2,4-diaminopyrimidines was designed and synthesized.

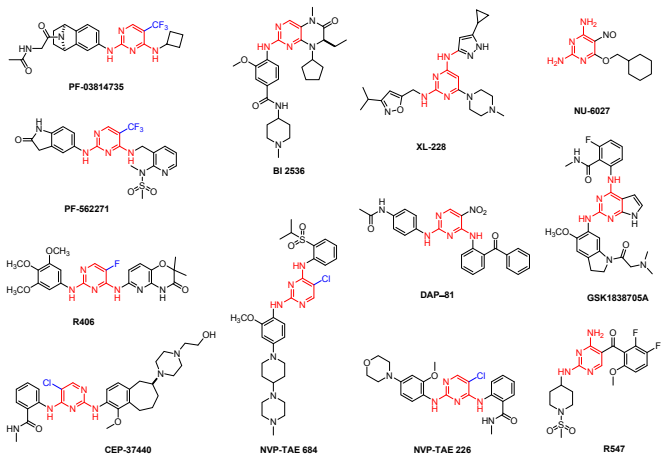
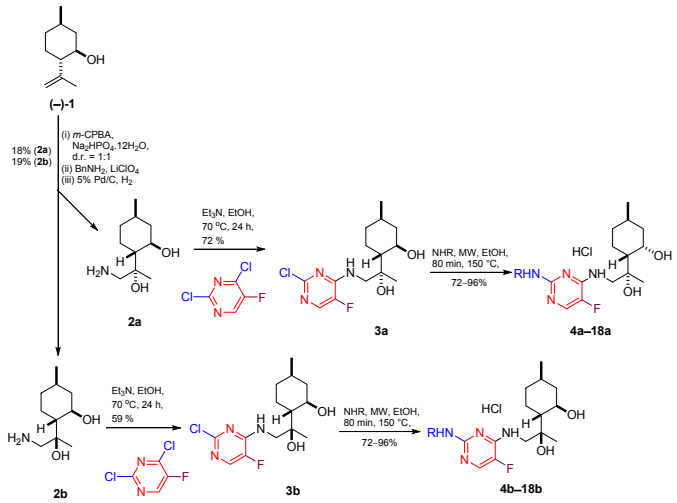


Figure 1. Some 2,4-diaminopyrimidine-based kinase inhibitors.

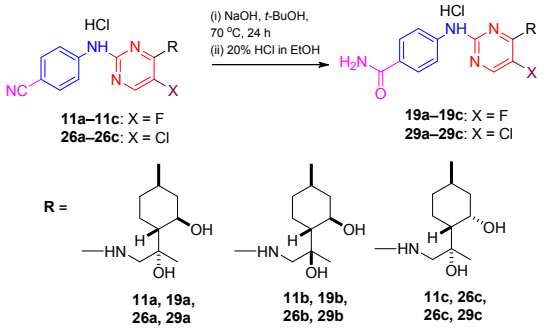
Results and discussion

The synthetic routes started with the preparation of aminodiols **2a–b** as key intermediates. Aminodiols **2a–b** were obtained from commercially available (–)-isopulegol **1** by a three-step sequence including epoxidation with *m*-CPBA followed by ring-opening of the corresponding oxiranes with benzylamine and subsequent hydrogenolysis on 5 % Pd/C.[93] The (–)-isopulegol-derived 2,4-diaminopyrimidines **4a**–**18a** were prepared as shown in Scheme 1 and Table 1 *via* the coupling of commercially available 5-fluoro-2,4-dichloropyrimidine with building block **2a** followed by microwave-assisted S_NAr coupling reactions with aniline derivatives in moderate to high yields (Scheme 1, Table 1).[92,94] The intermediate **2b** underwent a similar transformation to afford compounds **4b–18b** with a 5-fluoro substituent in the pyrimidine scaffold (Scheme 1, Table 1).



Scheme 1. (–)-Isopulegol-based 5-fluoro-2,4-diaminopyrimidines.

Attempts to directly synthesize **19a–b** from **3a–b** and *p*-aminobenzamide *via* microwave-assisted heating method were not successful despite of the elongated reaction time. Fortunately, this was achieved by hydrolysing their precursor nitriles **11a–b** under alkaline conditions.[95] The analogues **19a–b** were obtained in satisfactory yields as final products (Scheme 2).



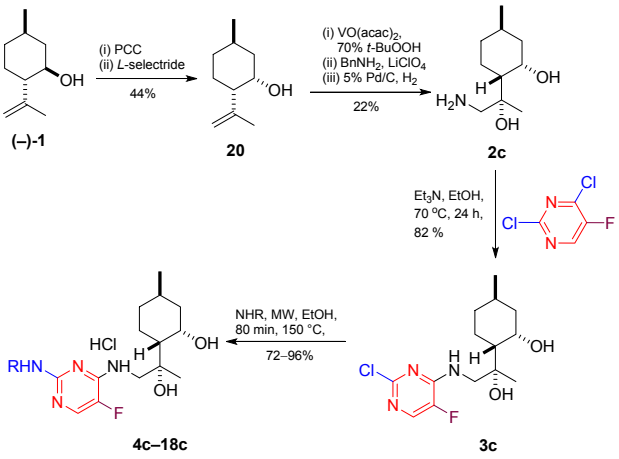
Scheme 2. Preparation of 2,4-diaminopyrimidine carboxamide.

Table 1. (–)-Isopulegol-based 5-fluoro-2,4-diaminopyrimidines.

Entry	Compound	R	Yield (%)
1	**4a**	4-Trifluorophenyl	58
2	**4b**		72
3	**4c**		58
4	**5a**	4-Carboxyphenyl	60
5	**5b**		60
6	**5c**		53
7	**6a**	4-(Methoxy-carbonyl)phenyl	73
8	**6b**		76
9	**6c**		66
10	**7a**	4-(Ethoxycarbo-nyl)phenyl	85
11	**7b**		71
12	**7c**		57
13	**8a**	4-Acetamidophenyl	73
14	**8b**		59
15	**8c**		59
16	**9a**	4-Hydroxyphenyl	82
17	**9b**		82
18	**9c**		65
19	**10a**	4-Methoxyphenyl	79
20	**10b**		63

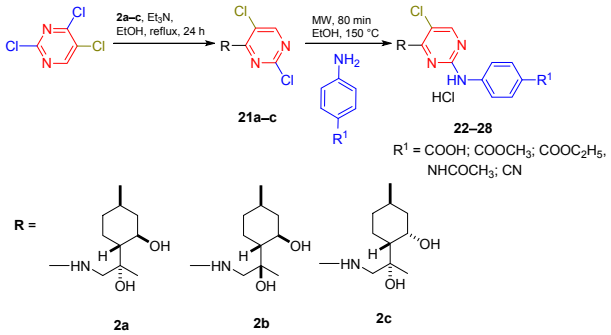
21	**10c**		71
22	**11a**	4-Cyanophenyl	40
23	**11b**		48
24	**11c**		40
25	**12a**	4-Nitrophenyl	23
26	**12b**		15
27	**12c**		15
28	**13a**	4-Morpholinophenyl	83
29	**13b**		55
30	**13c**		69
31	**14a**	4-(4-Methylpiperazin-1-yl)phenyl	34
32	**14b**		34
33	**14c**		27
34	**15a**	4-Sulfamoylphenyl	70
35	**15b**		56
36	**15c**		42
37	**16a**	Phenyl	67
38	**16b**		75
39	**16c**		50
40	**17a**	Pyridinyl	53
41	**17b**		44
42	**17c**		44
43	**18a**	1-Methyl-1*H*-pyrazol-4-yl	93
44	**18b**		84
45	**18c**		76

Besides the effects of the stereochemistry on aminoalcohol moiety, the antiproliferative activity also depends on the stereochemical aspects of the hydroxy substituent on the cyclohexyl core.[92,94] Therefore, the synthesis of library **4c–18c** was performed using the synthetic routes and protocols shown in Scheme 3. The preparation of building block **2c** was achieved from (+)-neoisopulegol **20**, obtained from **1** in two steps by Jones oxidation of the hydroxy group, followed by stereospecific reduction of (5)-isopulegone over a stoichiometric amount of L-Selectride into the desired *cis* diastereoisomer,[96] according to literature-reported protocols[97] as mentioned in the Scheme 3. The starting material **2c** was lately reacted with 5-fluoro-2,4-dichloropyrimidine to obtain intermediate **3c** in high yield. The displacement of the 4-chloro group on the 2,4-dichloropyrimidine moiety by various anilines provided the final targets **4c–18c** with moderate to excellent yields (Scheme 3, Table 1).[92] The hydrolysis of nitrile **11c** in alkaline condition led to the formation of carboxamide **19c** in moderate yield (Scheme 2).



Scheme 3. (+)-Neoisopulegol-based 5-fluoro-2,4-diaminopyrimidines.

The difference between halogen atom at 5-carbon position on pyrimidine ring could affect the electron-withdrawing properties improving *in vitro* antiproliferative activity.[54] The synthesis of library **22–26** with 5-chloro substituents on the pyrimidine skeleton was described in Scheme 4. The key intermediates **21a–c** were synthesized from commercially available 2,4,5-trichloropyrimidine and building blocks **2a–c** using literature-reported protocols.[92] In the same manner, these intermediates were directly reacted *via* nucleophilic aromatic substitution with aniline derivatives to obtain the final library **22–28** possessing chlorine in the 5-position of the pyrimidine skeleton in moderate yields (Scheme 4, Table 2). Treatment of nitriles **26a–c** with powdered potassium hydroxide in *tert*-butyl alcohol gave benzamides **29a–c** (Scheme 2) in good yields.[95]



Scheme 4. (–)-Isopulegol-based 5-chloro-2,4-diaminopyrimidines.

Table 2. (–)-Isopulegol-based 5-chloro-2,4-diaminopyrimidines.

Entry	Compound	R^1	Yield (%)
1	**22a**	4-Carboxyphenyl	49
2	**22b**		31
3	**22c**		38
4	**23a**	4-(Methoxycarbo-nyl)phenyl	50
5	**23b**		45
6	**23c**		45
7	**24a**	4-(Ethoxycarbo-nyl)phenyl	54
8	**24b**		43
9	**24c**		51
10	**25a**	4-Acetamidophenyl	57
11	**25b**		56
12	**25c**		52
13	**26a**	4-Cyanophenyl	46
14	**26b**		32
15	**26c**		43
16	**27a**	4-Morpholinophenyl	64
17	**27b**		28
18	**27c**		36
19	**28a**	4-(4-Methylpiperazin-1-yl)phenyl	52
20	**28b**		31
21	**28c**		27

Cytotoxic effect

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DOI: 10.1039/D6MD00278A

Since several 2,4-diaminopyrimidines exhibited significant cytotoxicity on colorectal cancer cell lines,[70,91] the *in vitro* cytotoxic effects of the prepared isopulegol-based 2,4-diaminopyrimidine analogues were also screened on human colon adenocarcinoma lines, including Colo205 and Colo320 by MTT assay. Doxorubicin (DOX) and Cisplatin (CIS), clinically applied anticancer agents, were used as reference compounds, and the results were summarized in Table 3 and Table 2 in Supporting information.

Table 3. Cytotoxic activity of 2,4-diaminopyrimidines on Colo 205 and Colo 320 colon adenocarcinoma cell lines and MRC-5 normal lung fibroblast cell line.

Compound	IC_{50} (μM)		
	Colo 205	Colo 320	MRC-5
4b	3.38±0.06	2.09±0.17	1.72±2.35
5b	>100	>100	>100
6a	2.24±0.36	1.47±0.03	>100
6b	1.20±0.24	0.46±0.02	<0.19
6c	7.18±0.68	12.63±0.45	>100
7a	13.75±1.46	12.97±1.96	<0.19
7b	1.03±0.02	1.29±0.24	>100
7c	34.33±1.85	20.54±0.69	>100
8b	4.52±0.4	6.99±0.47	2.46±2.92
9b	7.11±0.13	3.53±0.19	3.62±4.94
10b	1.56±0.34	1.7±0.03	0.95±0.86
11b	0.99±0.04	0.51±0.06	0.52±0.68
12b	2.34±0.24	2.01±0.07	<0.19
13b	1.46±0.13	1.71±0.19	0.79±0.95
14b	0.98±0.04	0.88±0.03	0.51±0.66
15b	14.58±1.47	>100	8.03±9.27
16b	5.78±0.39	5.56±0.17	<0.19
17b	>100	>100	>100
18b	16.3±1.16	11.53±1.29	8.73±10.7
19b	7.91±0.78	6.89±0.23	>100
22b	14.75±0.22	8.29±0.82	>100

23b	1.03±0.04	0.8±0.15	0.53±0.7
24b	0.75±0.04	0.8±0.09	0.39±0.5
25b	0.52±0.02	0.47±0.01	0.27±0.36
26b	1.25±0.1	0.88±0.04	0.67±0.82
27b	0.64±0.05	0.38±0.02	0.35±0.41
28b	0.26±0.02	0.3±0.01	0.14±0.17
DOX	1.85±0.77	3.96±0.66	>8.62
CIS	14.52±0.79	16.46±1.67	85.39±1.78

The results indicated that 2,4-diaminopyrimidine derivatives **6a** (SI > 44), **6c** (SI > 7), **7b** (SI > 77) and **19b** (SI > 12) exhibited potent inhibitory properties on doxorubicin-sensitive Colo 205 and doxorubicin-resistant Colo 320 adenocarcinoma cell lines comparable to those of the reference agent, doxorubicin and cisplatin, with no cytotoxicity against fibroblasts. Eight compounds (**11b, 14b, 24b, 25b, 25c, 27b, 27c, 28b**) showed considerable cytotoxic action, with IC_{50} values below 1 μM (SI < 1). The structure activity relationship (SAR) was investigated as below

Compound **7b** displayed a high potency in the range of the most active cytotoxic agents (SI > 77). Replacement of *p*-ethyl ester function in **7b** by *p*-methyl ester, cyano or methylpiperazin-1-yl as shown in **6b**, **11b** or **14b**, respectively, retained the activity, in contrast, resulted in over 200-fold toxicity in fibroblasts (SI < 1), emphasizing the importance of the ethyl ester moiety. In addition, the 2–5-fold loss of inhibitory activity was also observed when the substitution of the ethyl ester group in **7b** was accomplished by trifluoromethyl (**4b**), acetamido (**8b**), methoxy (**10b**), nitro (**12b**), morpholino (**13b**), or even by hydrogen (**16b**). In all cases, these replacements led to much less selectivity between cancer and normal cell lines (SI < 1) than the parent molecule (**7b**). Compound **19b** (SI > 12), where aniline ring has a carbamoyl substituent in the *para* position, was 8-fold less active than compound **7b**, whereas introduction of *p*-COOH in aniline portion (compound **5b**) resulted in the total loss of *in vitro* potency. These observations suggested that the *p*-$COOC_2H_5$ moiety on the phenyl ring was important to maintain the inhibitory activity. Furthermore, the activity of **16b** (SI < 0.03) was significantly higher than **17b** and **18b** (SI < 1), indicating the importance of phenyl ring for cytotoxic effects. Next, the effect of halogen atom in the pyrimidine ring was studied, and the results indicated that *in vitro* cytotoxicity upon treatment of the two cancer cell lines and fibroblasts of compounds **22b–28b** substituted with chloride at C-5 of pyrimidine showed more potent effects compared with compounds **4b–19b**, in which C-5 in pyrimidine was substituted with fluorine, respectively (Table 3). The influences of stereochemistry of hydroxy group in cyclohexyl ring were analysed and the results were presented that in case of 5-fluoropyrimidine, hydroxy group with *R* configuration (**6a, 7a**) was found to be more effective than its corresponding isomer (**6c, 7c**) whereas the replacement of 5-fluoro by 5-chloro in pyrimidine system, (*S*)-isomer (**23c**, **24c**) showed more potent effect compared than (*R*)-isomer (**23a**, **24a**). From the results of the *in vitro* cytotoxic assay, it was found that compounds (**6b, 7b, 23b, 24b**) with (*R*)-OH substituent in aminoalcohol scaffold showed more potent cytotoxic effects in comparison to (*S*)-analogue (**6a, 7a, 23a, 24a**).

Molecular docking analysis

To elucidate the binding mechanisms and verify the consistency between the structural features and potential inhibitory activity, molecular docking simulations were performed to predict the interactions of compounds **6a, 6c, 7b, 19b, 25b** and **28b** with the Aurora kinase A receptor (PDB: 4DEE). As summarized in Figure 2 as well as Figure 1 and Table 2 in supporting information, these derivatives occupy the ATP-binding pocket through distinct electrostatic and hydrophobic networks.

Compound **7b** establishes a stable binding pose anchored by hydrogen bonds to Asn261 and Glu260, alongside a critical metal-acceptor interaction with Mg501 and a halogen (fluorine) bond to Gly142. This stability is further reinforced by π-anion interactions with Glu260 and a dense van der Waals shell involving Leu139, Val174, Phe144, and Trp277. Notably, the binding of **7b** is tightly packed, though it exhibits some unfavourable donor-donor interactions with Lys143 and acceptor-acceptor repulsion with Asp274.

In contrast, compound **6a** displays a shifted hydrogen bonding network, engaging Asp274, Lys141, and Lys162, while maintaining the conserved metal-acceptor contact with Mg501. Unlike **7b**, compound **6a** lacks the specific halogen bond to Gly142 and faces steric challenges, indicated by an unfavourable bump with Mg502, which may constrain its conformational fit compared to its analogues. Its hydrophobic enclosure is primarily supported by π-alkyl interactions with Lys143 and van der Waals contacts with residues such as Ala273, Leu164, and Val147.

Compound **6c** preserves the hydrogen bond to Lys141, Lys162 and expands its halogen bonding to Gly142, it suffers from significant electrostatic mismatches. Specifically, **6c** is destabilized by unfavourable donor-donor and metal-donor repulsions with Lys143 and Mg ion, respectively, suggesting a less optimal fit despite its broad van der Waals footprint.

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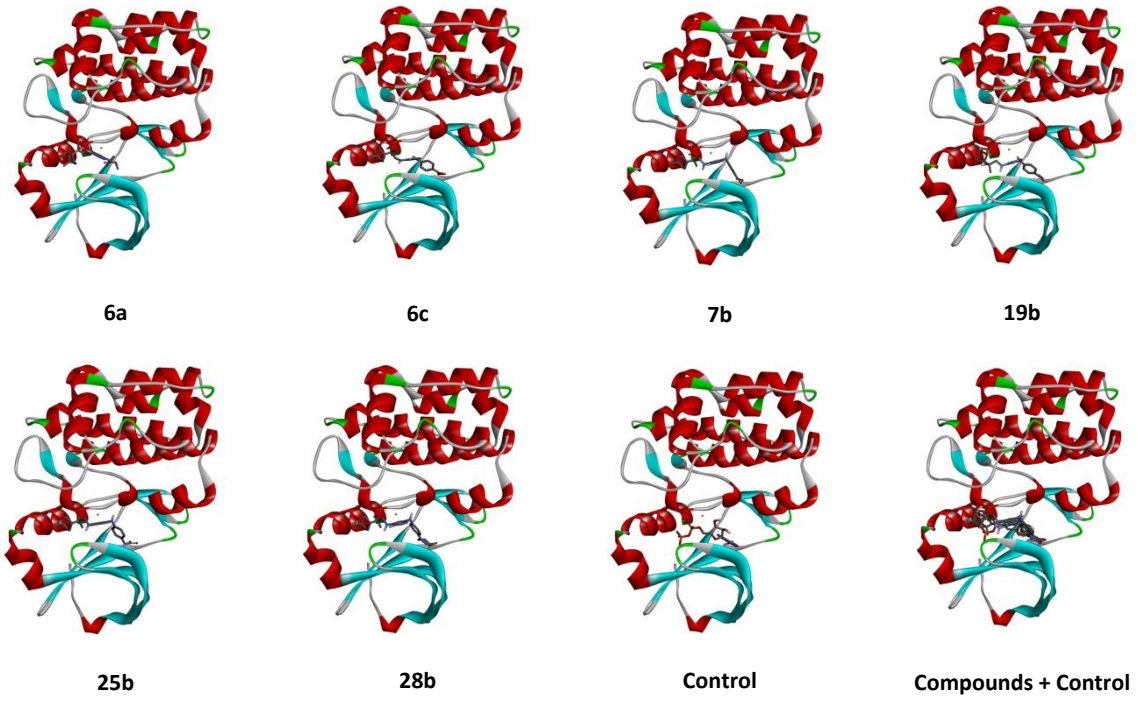

Figure 2. The 3D docking poses of Aurora A inhibition (ID: 4DEE) with 2,4-diaminopyrimidines

Compound **25b** appears to integrate key favourable features of the series, forming a stabilising hydrogen bond triad with Asp274, Asn261, and Glu260. Similar to **7b**, it utilizes a fluorine-mediated halogen bond with Gly142 and a π-anion interaction with Glu260, effectively bridging the binding motifs observed in the other ligands. Compound **28b**, while sharing the halogen bond at Gly142 and hydrogen bonds at Asp274 and Asn261, is penalised by multiple unfavourable interactions, including donor-donor clashes at Lys143 and acceptor-acceptor repulsion at Asp274.

Furthermore, compound **19b** exhibits a binding mode that is consistent with the interaction patterns established for the previously analysed compounds, particularly mirroring the stability seen in **25b**. It demonstrates a robust interaction profile anchored by a dual hydrogen-bonding network with Lys143 and Lys162. Its orientation is further secured by a metal-acceptor contact with Mg ion and a halogen bond to Lys141, while a dense hydrophobic shell, comprising π-σ interactions with Leu263 and π-alkyl contacts with Ala273, reinforces the binding.

In summary, the docking results indicate that halogen bonding and metal coordination with Mg ions serve as the key anchoring interactions within the Aurora A active sites. These ligands **7b**, **19b** **25b** and **28b** emerge as the most promising candidates by their ability to maintain these critical interactions effectively, with **28b** achieving high affinity (-9.8 kcal/mol) despite the presence of minor unfavourable electrostatic interactions. In contrast, **6a** and **6c** show reduced binding performance due to specific structural conflicts: a steric clash with the Mg ion in **6a**, while cumulative donor-donor or metal-donor repulsions in **6c**. Ultimately, these interaction profiles identify **25b** as the most balanced derivative, while **28b** remains a high-affinity analogue of interest. These results also explain the most effective cytotoxicity of adducts **25b** and **28b** in the *in vitro* assay.

***In silico* ADME prediction**

As summarised in Table 2–4 in Supporting information, the pharmacokinetic properties of derivatives **6a, 6c, 7b, 19b, 25b** and **28b** were estimated via the ADMETlab 2.0 web tool. All synthesized analogues adhered to Lipinski's Rule of Five with zero violations,

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exhibiting physicochemical properties conducive to drug-likeness. Furthermore, unlike many early-stage leads, these compounds displayed high gastrointestinal absorption and moderate Caco-2 permeability values ranging from -4.96 to -5.56, suggesting favourable oral bioavailability.

Metabolic stability was assessed against the cytochrome P450 (CYP) superfamily, the primary enzyme system responsible for the oxidation of xenobiotics in the liver and intestines. The data reveal a noticeable divergence in metabolic liability. Compounds **6a**, **6c** and **7b** act as broad-spectrum inhibitors, showing inhibitory activity against all major isoforms, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Conversely, **19b**, **25b** and **28b** demonstrated improved selectivity. Notably, **19b** and **25b** spared CYP1A2 and CYP2C19, while **28b** exhibited the most selective CYP inhibition profile, inhibiting only CYP3A4 while remaining non-inhibitory toward CYP1A2, CYP2C19, CYP2C9, and CYP2D6. In summary, while **6a, 6c** and **7b** present a higher potential for clinically meaningful drug-drug interactions (DDIs), the refined profiles of **19b**, **25b** and especially **28b** suggest a lower risk of metabolic interference upon co-administration with other therapeutics.

***In silico* toxicity profiles**

Additionally, the safety profiles of the analogues were predicted using the Deep-PK computational tool (Table 2-4 in Supporting information). In terms of acute toxicity, compound **25b** suggested the most favourable acute toxicity profile with an LD_{50} of 2,888 mg/kg, classifying it within GHS Category 5, which is low toxicity.[98] In contrast, **6a** (1,498 mg/kg), **6c** (1,643 mg/kg), **7b** (1,547 mg/kg), **28b** (1,213 mg/kg) fall into GHS Category 4.[98] Compound **19b** exhibited the highest acute toxicity risk with an LD_{50} of 832 mg/kg.

Regarding organ-specific endpoints, the toxicity estimation for **6a, 6c, 7b** and **25b** predicted safety concerning carcinogenesis. However, compounds **19b** and **28b** were flagged for potential carcinogenesis, which may compromise their developability. All compounds in the series were predicted as safe for drug-induced liver injury type I (DILI) but were flagged for potential liver injury type II and micronucleus formation, suggesting a potential risk of hepatotoxicity and genotoxicity that necessitates monitoring in downstream assays.

Mechanism-based toxicity screening revealed that while all compounds are predicted to be safe for the Androgen, Estrogen, including their LBDs, and Thyroid receptors, there is a divergence in Glucocorticoid Receptor (GR) interaction. Compounds **6a, 6c, 7b** and **25b** were predicted as GR toxic, suggesting potential GR-mediated off-target effects. Interestingly, compounds **19b** and **28b** were the sole analogues predicted as GR safe. Finally, all six compounds showed a potential hERG liability, a common signal in kinase inhibitor discovery. Nevertheless, the favourable acute safety profile of **25b** marks it as the most promising lead for further toxicological optimization.

Mechanism-linked SAR

To further rationalize the observed structure–activity relationship (SAR) trends, the cytotoxicity data were interpreted in conjunction with molecular docking results within the ATP-binding pocket of Aurora kinase A (PDB: 4DEE).

The superior activity and selectivity of compound **7b** can be mechanistically explained by its ability to establish a multi-point anchoring network within the binding pocket. Docking analysis revealed that the para-ethyl ester substituent plays a crucial role in mediating hydrogen bonding interactions with key residues such as Asn261 and Glu260, thereby stabilizing the ligand orientation toward the hinge region. In addition, this group enhances complementarity within the hydrophobic pocket formed by residues including Leu139, Val174, and Phe144. In contrast, replacement of the ester group with more polar functionalities such as –COOH or less optimally oriented substituents such as –CN and $–CONH_2$ disrupts this interaction network, leading to reduced binding stability through the loss of key hydrogen bonds or increased off-target interactions. These effects are consistent with the experimentally observed loss of selectivity (SI < 1). Therefore, the para-ethyl ester moiety can be regarded as a dual-function pharmacophore that balances hydrogen bonding and hydrophobic interactions to achieve optimal binding and selectivity.

The nature of the halogen substituent at the C-5 position of the pyrimidine ring also plays a critical role in modulating ligand–protein interactions. Fluorinated derivatives, exemplified by compound **7b**, were found to form halogen bonding interactions with Gly142, which contribute to proper positioning within the ATP-binding site while maintaining a favorable selectivity profile. In contrast, substitution with chlorine, as observed in the **22–28** series, enhances hydrophobic contacts and overall binding affinity, which is reflected by lower IC_{50} values. However, this increased hydrophobicity also promotes non-specific interactions and reduces discrimination between cancer and normal cells, resulting in diminished selectivity. These findings suggest that fluorine provides a more balanced interaction profile, whereas chlorine favours stronger but less selective binding.

Another important feature observed among active compounds such as **7b**, **25b**, and **28b** is their ability to interact with the Mg^{2+} ion present in the active site, forming metal-acceptor interactions that further stabilize ligand binding. Moreover, hydrogen bonding interactions with hinge region residues, particularly Glu260 and Asn261, mimic the canonical binding mode of ATP-competitive kinase inhibitors. However, less active compounds such as **6a** and **6c** exhibit steric clashes with the Mg^{2+} ion and unfavourable donor–donor interactions, for instance with Lys143, which likely

compromise binding efficiency and account for their reduced biological activity.

The stereochemistry of the hydroxy substituent on the cyclohexyl ring also significantly influences ligand binding. The *R*-configuration, as observed in compound **7b**, enables optimal spatial alignment of the aminoalcohol moiety toward polar residues within the binding pocket, facilitating the formation of stable hydrogen bonding networks without introducing steric hindrance. In contrast, the corresponding *S*-isomers tend to misalign key functional groups and may introduce electrostatic repulsion or steric clashes, thereby reducing binding affinity. This stereochemical dependence highlights the importance of three-dimensional complementarity between the ligand and the protein binding site.

Overall, the biological activity of these 2,4-diaminopyrimidine derivatives is governed by a combination of hinge-binding hydrogen bonds with residues such as Glu260 and Asn261, halogen bonding interactions with Gly142 for positional stabilization, metal coordination with Mg^{2+}, and hydrophobic enclosure within the ATP-binding pocket. Among the series, compound **7b** achieves the most favorable balance of these interactions, which explains its superior potency and selectivity. In contrast, highly potent but non-selective compounds such as **25b** and **28b** likely derive their activity from increased binding affinity at the expense of specificity. These findings suggest that fine-tuning the balance between polar anchoring interactions and hydrophobic complementarity within the Aurora A kinase binding pocket is critical for achieving both potency and selectivity in this scaffold.

Conclusions

Aurora kinases have been of interest as potential therapeutic targets. In the current investigation, a series of (–)-isopulegol-derived 2,4-diaminopyrimidines that exert their cytotoxic effects in a panel of colorectal cancer cell lines was designed and synthesized.

All of the compounds were screened for their cytotoxic effects on human colon adenocarcinoma lines. Compounds **6a** and **7b** exerted outstanding activities against the malignant cells with no action on fibroblasts (SI > 44), indicating considerable cancer selectivity. The structure–activity relationship clearly indicated the importance of stereochemistry on the cyclohexyl ring and aminodiol moiety, as well as the effects of substituents on the pyrimidine scaffold.

The molecular docking studies revealed that the ester group at the para position of the aniline moiety in compounds **6a** and **7b** played a crucial role in binding with Aurora A kinase. These data together suggested the potential of **6a** and **7b** as these promising therapeutic candidates for addressing colorectal cancer based on Aurora A kinase inhibition.

Author contributions

T.L.M: Writing – original draft, validation, methodology, investigation, N.S: methodology, investigation, formal analysis investigation, M.C.N: Investigation, H.N.K.T: Writing – original draft, formal analysis, G.S: review & editing, formal analysis, data curation, K.M.T: methodology, review & editing, Z.S: supervision, resources, funding acquisition.

Conflicts of interest

There are no conflicts to declare

Data availability

Data supporting this article have been included as part of the Supplementary Information and available therein

Acknowledgements

We are grateful for Hungarian Research Foundation (OTKA No. K-138871) and the Ministry of Human Capacities, Hungary, grant TKP-2021-EGA-32; and the University of Szeged Open Access Fund, Grant ID: 8601.

The high-resolution mass spectrometric analysis was performed by Robert Berkecz. Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

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View Article Online
DOI: 10.1039/D6MD00278A

Data availability statements

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We declare that the data supporting this article have been included as part of the Supplementary Information and available therein.

Sincerely yours,

Prof. Zsolt Szakonyi Ph.D.
corresponding author