Bioorganic & Medicinal Chemistry Letters 22 (2012) 1317–1321

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of benzo[*d*]imidazole derivatives as potential anti-cancer agents

Hamad M. Alkahtani, Abdullahi Y. Abbas, Shudong Wang *

School of Pharmacy and Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK

ARTICLE INFO

Article history:
Received 17 October 2011
Revised 14 December 2011
Accepted 16 December 2011
Available online 23 December 2011

Keywords:
RNA polymerase-II
Cyclin-dependent kinase inhibitor
Apoptosis
Anti-cancer therapy

ABSTRACT

We herein report the synthesis, biological activity and structure–activity relationship of derivatives of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole and benzo[*d*]imidazole. A lead compound **6o** demonstrates potent anti-proliferative activity and the ability to induce cancer cell apoptosis.

Despite more than 13 members having been identified, the cyclin-dependent kinases (CDKs) can generally be divided into two groups based on roles in cell-cycle progression and transcriptional regulation. Association of CDK1, CDK2, CDK4, and CDK6 with A-, B-, E-, and D-type cyclins ensures an orderly and controlled progression of the cell proliferation cycle. CDK7 and CDK9, on the other hand, are primarily involved in the regulation of transcription through phosphorylation of the C-terminal domain (CTD) of RNA polymerase-II (RNAP-II).[1] De-regulation of various components controlling the cell-cycle plays an essential role in tumor pathogenesis. This has promoted the development of pharmacological small-molecule CDK inhibitors that can be used for cancer therapy. However, the fact that transformed cells depleted of cyclins and CDKs continue to proliferate[2] indicates that that the specific targeting of individual cell-cycle CDKs may not be an optimal therapeutic strategy due to functional redundancy.[1]

Flavopiridol, a pioneer pan-CDK inhibitor, has demonstrated marked efficacy in refractory and relapsed chronic lymphocytic leukemia (CLL).[3] This has been attributed to flavopiridol inhibition of CDK-mediated transcriptional regulation. CLL cells are mostly accumulated mature B cells which are resistant to apoptosis. Although strongly inhibiting most CDKs, the primary mechanism of action of flavopiridol is believed to be through inhibiting CDK9 and altering the transcription of apoptosis-related proteins in CLL. Flavopiridol is the most potent CDK9 inhibitor identified, and it has been shown that this inhibition is associated with a decline in short-lived anti-apoptotic proteins Mcl-1 and X-linked inhibitor of apoptosis (XIAP), which results in reinstatement of the ability of CLL cell to undergo apoptosis.[4]

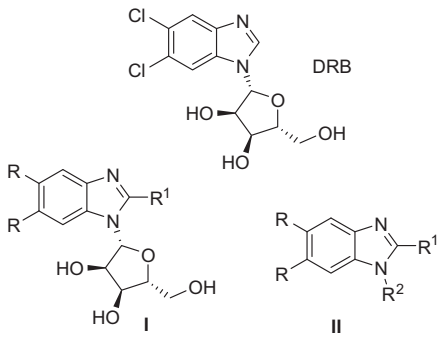


Figure 1. Structures of DRB and the designated derivatives.

5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) is an adenosine analog that has been shown to inhibit RNAPII transcription both in vitro and in vivo.[5,6] It acts by competing with ATP[7] and is believed to be the most selective CDK9 inhibitor available to date.[1] It inhibits CDK9 potently with an IC_{50} value of 600 nM by biochemical assay and shows excellent selectivity against other CDKs. However, this striking selectivity is not associated with the level of cellular potency essential for anti-cancer agents.

Our interest in developing drugs that target transcriptional CDKs has resulted in the discovery of several pre-clinical and clinical candidates for cancer therapy.[8–13] In order to identify more selective CDK9 inhibitors we selected DRB as a model for chemical

* Corresponding author. Tel.: +44 1158466863; fax: +44 01159513412.
E-mail address: shudong.wang@nottingham.ac.uk (S. Wang).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.12.088

modification and expansion aimed at defining the structure activity relationship and improving cellular potency and drug-likeness by replacing the metabolically-liable ribose of DRB with an aryl or a carbocycle. Accordingly, we prepared a class of DRB and benzo[*d*]imidazole derivatives (Type-I and Type-II, Fig. 1). In this report, we describe the synthesis and biological evaluation of these compounds. The initial structure–activity relationship analysis provided invaluable guidance in progressing our CDK9-targeted drug discovery programmes.

The synthetic chemistry employed to prepare Type-I and -II compounds is outlined in Scheme 1. Condensation reactions between benzene-1,2-diamine **1** and the appropriate carboxylic acids yielded benzo[*d*]imidazoles **2a–g**[14–16] where various substitutions, including alkyls and halides, were introduced at 2C-position of the imidazole ring system; that is, R^1 to investigate their effects on biological activity. The preparation of o-acetylated nucleosides **3** involved the formation of silylated benzimidazole by treating **2** with bis(trimethylsilyl)acetamide (BSA) in MeCN, followed by condensation with acylated sugar under Vorbrüggen's conditions[17] in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf). Subsequent hydrolysis of the latter in methanolic ammonia resulted in benzo[*d*]imidazole nucleosides **4a–d** in high yield.

Modification of the nucleoside moiety with several acyclic and cyclic systems resulted in compound class Type-II (Fig. 1). Thus treatment of benzene-1,2-diamines **1** with either 2-chlorobenzaldehyde or 2-florobenzaldehyde yielded the corresponding **5a**[18] and **5b**. Diversity of 1N-substituted benzo[*d*]imidazoles **6a–k** and **6n–r** were obtained by alkylation reactions between 1*H*-benzo[*d*]imidazoles **2a, 2d–g** and the appropriate alkyl halides. Final reduction of the nitrobenzene moieties of **6i–j** in the presence of $SnCl_4$ under acidic conditions afforded the respective anilino derivatives **6l–m**.

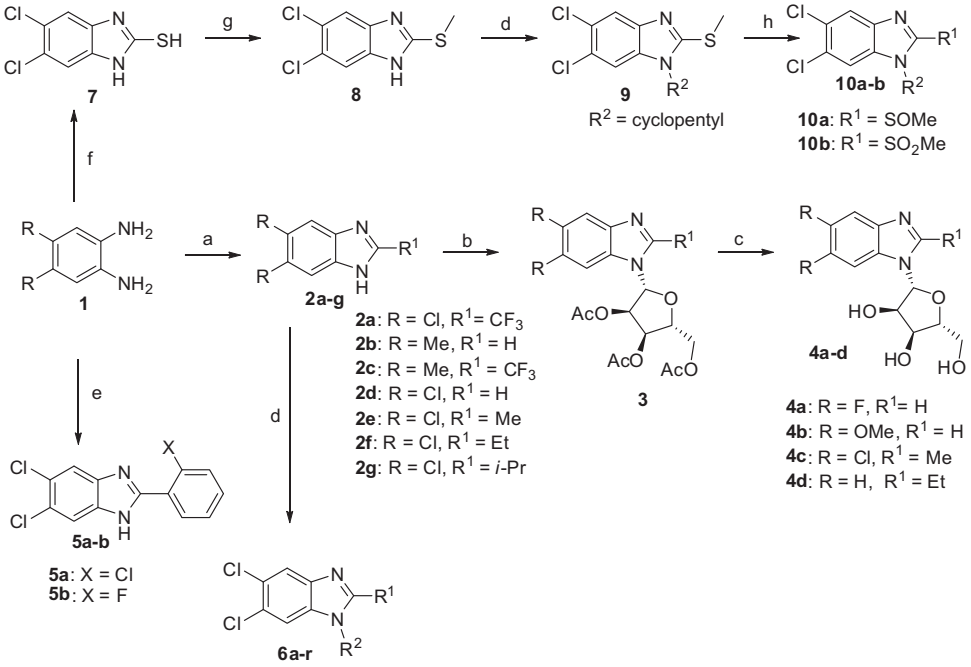
To further probe the electron donating and withdrawing effects thioether, sulfinyl and sulfonyl substituent were introduced to 2C-position of benzo[*d*]imidazole. 4,5-Dichlorobenzene-1,2-diamine **1** was heated with carbon disulfide in ethanolic potassium hydroxide, followed by methylation to give **8**,[19] which was in turn treated with chlorocyclopentane to afford 5,6-dichloro-1-cyclopentyl-2-(methylthio)-1*H*-benzo[*d*]imidazole **9**. Methylsulfinyl- (**10a**) and methylsulfonyl-1*H*-benzo[*d*]imidazole (**10b**) were obtained by oxidation in the presence of *m*-CPBA.

Anti-proliferative activity of selected compounds was assessed against HCT-116 colorectal carcinoma and MCF-7 breast carcinoma cells using a standard 48-h MTT cytotoxicity assay.[8] The results are summarized in Table 1.

Replacement of the 5,6-dichloride group of the benzimidazole nucleus with either fluoride (**4a**: R = F)[20] or methoxide (**4b**: R = OMe) in the context of 1-β-D-ribofuranosylbenzimidazole did not offer any superiority over DRB in terms of cytotoxic effects on the tumor cell lines tested. Similarly, introduction of a methyl or ethyl group at the R^1 in **4c**[21] or **4d** failed to enhance activity, irrespective of what substituent the benzene ring bears.

Surprisingly, compound **2a**[14] ($R^1 = CF_3$, R^2 = H, R = Cl) demonstrated good anti-proliferative activity in both HCT-116 and MCF-7 cells with GI_{50} of 7 and 6 μM respectively, being 3.5- and 6-fold more potent than DRB, suggesting that the ribose ring might be dispensable. The structural modification of **2a** by replacing 5,6-dichlorides with 5,6-dimethyl groups, that is, **2c**[14]: $R^1 = CF_3$, R = Me, or depleting trifluoromethyl (**2d**[15]: R^1 = H, R = Cl), however, dramatically reduced the activity compared with **2a**. Analogs with different substitutions at R^1, including alkyls (**2e**: R^1 = Me, **2f**: R^1 = Et, and **2g**: R^1 = *i*-Pr)[15,16] and substituted phenyls (**5a** and **5b**), in the context of 5,6-dichloro-1*H*-benzo[*d*]imidazole, had little effect on cell viability. The trifluoromethyl substitution was therefore identified as the most effective residue at the position R^1 of 5,6-dichlorid benzo[*d*]imidazole for cellular potency.

Keeping the portion of 5,6-dichloro-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazole while introducing a number of acyclic or cyclic systems at 1N-position led to **6a–e**. As shown in Table 1, although **6a**–**d**, where R^1 is alkyl; that is, Me (**6a**), Et (**6b**), *i*-Pr (**6c**) or Allyl (**6d**), caused a loss in anti-proliferative activity, 5,6-dichloro-1-



Scheme 1. Reagents and conditions: (a) R^1COOH, rf; 4 h, 70–98%, or R^1COOH, Discovery Microwave, 145 °C; 20 min; (b) (i) BSA, MeCN, reflux, 1 h, (ii) (*2S,3R,4R,5R*)-5-(acetoxymethyl)tetrahydrofuran-2,3,4-triyl triacetate in MeCN, TMSOTf, reflux, 24 h, 19–50%; (c) 7 M NH_3 in MeOH, rt, 24 h, 100%; (d) 2a, 2d, 2e, 2f, 2 g, or 8; R^2X, NaOH, MeCN, 24 h, 1–70%; (e) ArCHO, $Na_2S_2O_5$, EtOH, reflux, 38–86%; (f) CS_2, KOH, EtOH, reflux, 4 hr, 87%; (g) MeI, K_2CO_3, reflux, 4 h, 80%; (h) DCM, *m*-CPBA, −40 °C–rt, 70–100%.