Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3083–3086

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# 2,6-Disubstituted Pyran-4-one and Thiopyran-4-one Inhibitors of DNA-Dependent Protein Kinase (DNA-PK)

Jonathan J. Hollick,[a] Bernard T. Golding,[a] Ian R. Hardcastle,[a] Niall Martin,[b] Caroline Richardson,[b] Laurent J. M. Rigoreau,[a,b] Graeme C. M. Smith[b] and Roger J. Griffin[a,*]

[a]*Northern Institute for Cancer Research, School of Natural Sciences-Chemistry, Bedson Building, University of Newcastle, Newcastle Upon Tyne NE1 7RU, UK*

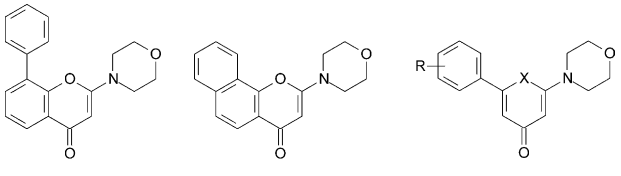
[b]*KuDOS Pharmaceuticals Limited, 327 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, UK*

Received 9 April 2003; accepted 16 May 2003

**Abstract**—6-Aryl-2-morpholin-4-yl-4*H*-pyran-4-ones and 6-aryl-2-morpholin-4-yl-4*H*-thiopyran-4-ones were synthesised and evaluated as potential inhibitors of the DNA repair enzyme DNA-dependent protein kinase (DNA-PK). Several compounds in each series exhibited superior activity to the chromenone LY294002, and were of comparable potency to the benzochromenone NU7026 ($IC_{50} = 0.23$ μM). Importantly, members of both structural classes were found to be selective inhibitors of DNA-PK over related phosphatidylinositol 3-kinase-related kinase (PIKK) family members. A multiple-parallel synthesis approach, employing Suzuki cross-coupling methodology, was utilised to prepare libraries of thiopyran-4-ones with a range of aromatic groups at the 3′- and 4′-positions on the thiopyran-4-one 6-aryl ring. Screening of the libraries resulted in the identification of 6-aryl-2-morpholin-4-yl-4*H*-thiopyran-4-ones bearing naphthyl or benzo[*b*]thienyl substituents at the 4′-position, as potent DNA-PK inhibitors with $IC_{50}$ values in the 0.2–0.4 μM range.


## Introduction

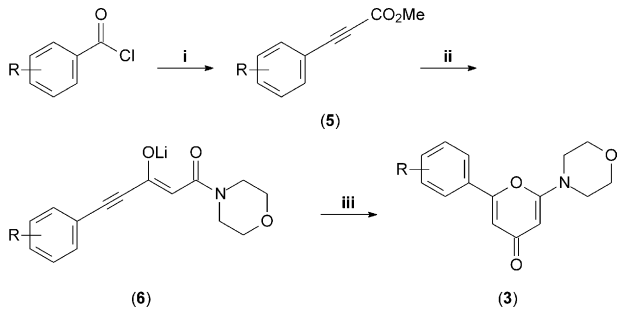
The nuclear serine/threonine protein kinase DNA-dependent protein kinase (DNA-PK), a member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family, comprises a large catalytic subunit (DNA-PKcs), and a heterodimeric subunit (Ku).[1–3] DNA-PK is activated and assembles at the site of DNA double strand breaks (DSBs), and is a crucial component of the cellular DNA DSB repair machinery.[4] Phosphorylation of a number of target proteins has been observed in vitro, including those involved in the cell cycle and apoptosis.[5] DNA-PK plays an essential role in the physiological process of V(D)J recombination,[2] and is also involved in the repair of DNA DSBs induced by ionising radiation and certain cancer chemotherapeutic agents. Importantly, cells without competent DNA-PK are hypersensitive to ionising radiation and radiomimetic agents, and inhibition of DNA-PK activity has been shown to potentiate the in vitro cytotoxicity of ionising radiation[5,6] and a number of anticancer drugs.[7–9] DNA-PK inhibitors may thus have clinical utility as radio- and chemo-potentiators in the treatment of cancer.




Although reportedly a selective ATP-competitive PI 3-kinase (PI 3-K) inhibitor ($IC_{50} = 1.5$–$2.0$ μM),[10,11] the chromenone LY294002 (**1**) has also been shown to exhibit comparable inhibitory activity against DNA-PK ($IC_{50} = 1.4$ μM, $K_i = 6.0$ μM).[12] As part of a research programme to develop potent and selective DNA-PK inhibitors suitable for eventual clinical evaluation, we have utilised **1** as a structural lead. In the absence of suitable crystal structure information for DNA-PK, a

*Corresponding author. Tel.: +44-191-222-8591; fax: +44-191-222-8591; e-mail: r.j.griffin@ncl.ac.uk

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00652-8



**Scheme 1.** General synthesis of pyran-4-ones. Reagents and conditions: (i) (a) $Ph_3P{=}CHCO_2Me$, PhMe, reflux; (b) 250 °C; (ii) *n*-BuLi, $(i\text{-Pr})_2NH$, *N*-acetylmorpholine, −78 °C→0 °C; (iii) $MeSO_3H$, 25 °C.

pharmacophore mapping approach has been exploited for the delineation of structure–activity relationships (SARs) around the core structure of **1**. These studies have resulted in the identification of a number of structurally diverse inhibitors, which are more potent and selective than **1**, as exemplified by NU7026 (**2**) ($IC_{50} = 0.23$ μM).[13] Further refinement of the pharmacophore model indicated that, whereas the 2-morpholin-4-yl substituent appears to be a prerequisite for inhibitory activity, 'minimalisation' of the chromenone core structure might be tolerated. In this paper we describe the synthesis and preliminary biological evaluation of DNA-PK inhibitors derived from the monocyclic pyran-4-one (**3**) and thiopyran-4-one (**4**) templates.

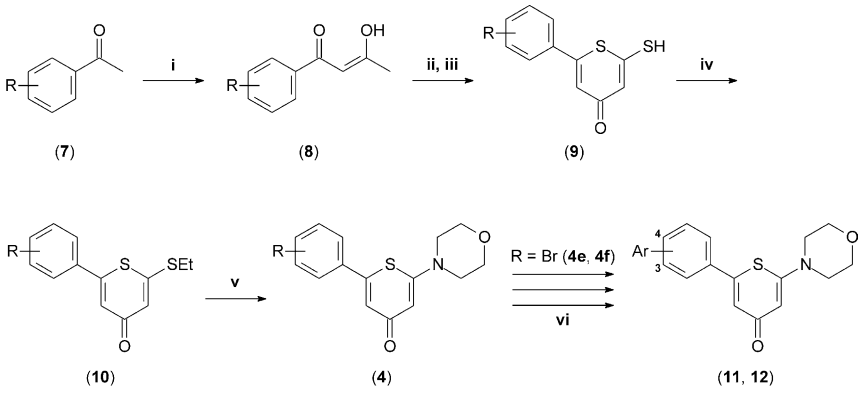
## Chemical Synthesis

The required 2,6-disubstituted pyran-4-ones (**3**) were prepared by a literature procedure as summarised in Scheme 1.[14] Briefly, treatment of the appropriate benzoyl chloride with methyl triphenylphosphoranylidine, gave the phenylpropiolate ester (**5**), which furnished the phenylacetylenic β-ketoamide (**6**) on reaction with the *O*-lithio derivative of acetylmorpholine. Ring closure of **6** to the required pyran-4-one (**3**) was achieved with methanesulfonic acid. To the best of our knowledge, the corresponding 6-aryl-2-morpholin-4-ylthiopyran-4-ones (**4**) have not been reported previously, and the synthesis of this heterocycle is summarised in Scheme 2. The substituted aroylacetone (**8**) was readily prepared by acylation of the appropriate acetophenone (**7**) with ethyl acetate–sodium ethoxide. Treatment of the dianion of **8** with carbon disulfide afforded, upon carefully controlled workup, the 2-mercapto-6-arylthiopyran-4-one (**9**), and alkylation of the thiol group of **9** with iodoethane gave **10** quantitatively. Final displacement of the 2-thioethyl substituent was effected by heating **10** with morpholine in ethane-1,2-diol to yield the target thiopyran-4-ones **4a**–**f**.[15]

Although unambiguous SARs are not apparent from the initial compounds synthesised, the nature and position of the 6-aryl substituent clearly influences DNA-PK inhibitory activity in both series (Table 1). In addition, preliminary in vitro studies with the pyran-4-ones (**3**) and thiopyran-4-ones (**4**) suggest that the latter series might have superior cellular activity, despite the slightly higher potency of the pyran-4-ones against DNA-PK. In order to investigate this further, a multiple-parallel synthesis (MPS) approach was utilised to generate two libraries with a range of aromatic groups at the 3′- and 4′-positions on the thiopyran-4-one 6-aryl ring. The required libraries were prepared by a Suzuki cross-coupling reaction between the 3′- and 4′-bromophenyl substituted thiopyran-4-ones (**4e** and **4f**), and a range of commercially available boronic acids and esters (Scheme 2).[16] A total of 97 thiopyran-4-one derivatives (42 from **4e** and 55 from **4f**) were deemed of suitable purity (≥85%) for evaluation in a pre-screen DNA-PK assay, from which six compounds emerged as sufficiently potent for $IC_{50}$ values to be determined (Table 2).

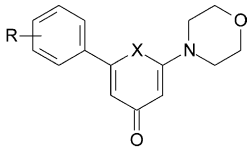
## Results and Discussion

Our previous studies have demonstrated that selective inhibition of DNA-PK, over other PIKK family members, is achievable through modification of the chromenone pharmacophore of **1**. For example, NU7026 (**2**) is approximately 6-fold more potent than **1** as a DNA-PK inhibitor, and at least 70-fold more selective for DNA-PK over PI 3-K (p110α), whereas **1** is essentially equipotent against both kinases.[13,17] However, the benzo[*h*]chromenone template of **2** is not readily amenable



**Scheme 2.** General synthesis of thiopyranones. Reagents and conditions: (i) NaOEt, EtOAc, THF, 25 °C; (ii) LDA, THF, $CS_2$, −78→25 °C; (iii) $H_2O$, 0 °C→25 °C, HCl (aq); (iv) $K_2CO_3$, EtI, $(Me)_2CO$, reflux; (v) morpholine, $(CH_2OH)_2$, 120→150 °C; (vi) $Pd(PPh_3)_4$, $K_2CO_3$, $ArB(OH)_2$, dioxane, 90 °C.

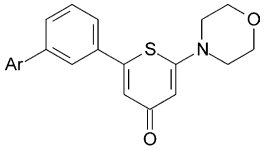
**Table 1.** Inhibition of DNA-PK by selected pyranone-4-ones and thiopyran-4-ones[a]



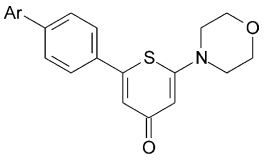

| Compd | X | R | $IC_{50}$ (μM)[b] | Compd | X | R | $IC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|
| **1** | — | — | 1.4 | **3g** | O | 4-MeO | 0.22 |
| **2** | — | — | 0.23 | **3h** | O | 4-$^t$Bu | 0.48 |
| **3a** | O | H | 1.10 | **4a** | S | H | 0.72 |
| **3b** | O | 3-F | 0.53 | **4b** | S | 4-Cl | 0.53 |
| **3c** | O | 4-F | 0.35 | **4c** | S | 4-MeO | 0.28 |
| **3d** | O | 4-Cl | 0.18 | **4d** | S | 4-$^t$Bu | 0.92 |
| **3e** | O | 2-MeO | 0.38 | **4e** | S | 3-Br | 0.80 |
| **3f** | O | 3-MeO | 0.54 | **4f** | S | 4-Br | 0.68 |

[a]Ref 17.
[b]Values are the means of at least three separate determinations.

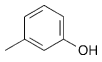
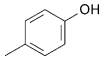
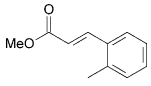
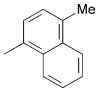
**Table 2.** DNA-PK-inhibitory activity of thiopyran-4-ones identified from library screens[a]




(**11**)




(**12**)

| Compd | Ar | $IC_{50}$ (μM)[a] | Compd | Ar | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **11a** |  | 0.92 | **12a** |  | 0.33 |
| **11b** |  | 0.35 | **12b** |  | 0.19 |
| **11c** |  | 0.84 | **12c** |  | 0.26 |

[a]Library compounds were assayed for % inhibition at 1.0, 0.5 and 0.1 μM. $IC_{50}$ values were determined for compounds exhibiting >50% inhibition at 1.0 μM, according to ref 17.

to extensive chemical modification, and in particular the introduction of substituents onto the fused aromatic ring system. Accordingly, it was envisaged that a 6-arylpyran-4-one or 6-arylthiopyran-4-one template, bearing the essential 2-morpholin-4-yl group, would offer more opportunities for introducing structural diversity in the aromatic region, while retaining the core pharmacophore elements common to **1** and **2**.

All of the pyran-4-ones (**3a**–**3h**) and thiopyran-4-ones (**4a**–**4f**) synthesised initially are more potent than LY294002 (**1**), with the pyran-4-ones generally tending to be at least equipotent (compare **3g** with **4c**) or slightly more potent (compare **3d** with **4b**, and **3h** with **4d**) than their thiopyran-4-one counterparts. An exception arises with the parent 6-phenyl derivatives, where the thiopyran-4-one (**4a**) is marginally more active than the corresponding pyran-4-one (**3a**). Although definitive SAR correlations are clearly not possible, it is evident that a lipophilic substituent at the 4′-position of the 6-aryl ring is favoured for both series, with two pyran-4-ones (**3d** and **3g**) and one thiopyran-4-one (**4c**) exhibiting activity comparable with NU7026 (**2**). Perhaps more importantly, the pyran-4-one/thiopyran-4-one template was found to retain the selectivity for DNA-PK observed for the benzo[*h*]chromenone scaffold. For example, **3g** was found to be at least 200-fold more potent an inhibitor of DNA-PK ($IC_{50}=0.22$ μM) compared with the other PIKK family members PI 3-K (p110α), ATM and ATR ($IC_{50}$ >50 μM). A similar degree of selectivity has also been observed in the thiopyran-4-one series.

Initial evaluation of the two thiopyran-4-one compound libraries (**11** and **12**) for DNA-PK inhibitory activity revealed a wide range of activities, with library members bearing an aryl substituent at the 4′-position (**12**) generally proving more potent than those with a 3′-aryl

group (**11**). Three compounds from each library (**11a**–**11c**) and (**12a**–**12c**) were identified from this pre-screen as being sufficiently potent to warrant $IC_{50}$ determinations (Table 2). Although there are no large differences in activity within this compound set, those thiopyran-4-ones with a 4′-aryl substituent (**12a**–**12c**) tend to be the more potent, consistent with the overall trend observed for the initial two libraries. Perhaps more interesting are the clear structural similarities between the 4′-aryl substituents on **12a**–**12c**, which comprise 1-naphthyl and 1-(4-methyl)naphthyl (**12b** and **12c**), and the isosteric 3-thianaphthene (3-benzo[*b*]thienyl) group (**12a**). These three thiopyran-4-ones exhibit DNA-PK inhibitory activity comparable with that of NU7026 (**2**), and are among the most potent DNA-PK inhibitors reported to date. In summary, we have established that pyran-4-ones and thiopyran-4-ones are versatile platforms for the development of potent and selective DNA-PK inhibitors, and have utilised a simple library approach for the identification of a potentially interesting class of inhibitors derived from the thiopyran-4-one pharmacophore. Further studies are underway to optimise the biological and pharmaceutical properties of this new series of DNA-PK inhibitors.

## Acknowledgements

The authors thank Cancer Research UK and the BBSRC (Studentship to J.J.H.) for financial support.

## References and Notes

1. Doherty, A. J.; Jackson, S. R. *Curr. Biol.* **2001**, *11*, R920.
2. Smith, G. C. M.; Jackson, S. P. *Genes Dev.* **1999**, *13*, 916.
3. Shiloh, Y. *Nat. Rev. Cancer* **2003**, *3*, 155.
4. Jackson, S. P. *Carcinogenesis* **2002**, *23*, 687.
5. Jackson, S. P. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 935.
6. Rosenzweig, K. E.; Youmell, M. B.; Palayoor, S. T.; Price, B. D. *Clin. Cancer Res.* **1997**, *3*, 1149.
7. Boulton, S.; Kyle, S.; Durkacz, B. W. *Eur. J. Cancer* **2000**, *36*, 535.
8. Kim, C. H.; Park, S. J.; Lee, S. H. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 753.
9. Oliveira, N. G.; Castro, M.; Rodrigues, A. S.; Gil, O. M.; Toscano-Rico, J. M.; Rueff, J. *Teratog. Carcinog. Mutag.* **2002**, *22*, 343.
10. Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F. *J. Biol. Chem.* **1994**, *269*, 5241.
11. Ward, S.; Sotsios, Y.; Dowden, J.; Bruce, I.; Finan, P. *Chem. Biol.* **2003**, *10*, 207, and references therein.
12. Izzard, R. A.; Jackson, S. P.; Smith, G. C. M. *Cancer Res.* **1999**, *59*, 2581.
13. Griffin, R. J.; Calvert, A. H.; Curtin, N. J.; Durkacz, B. W.; Golding, B. T.; Hardcastle, I. R.; Leahy, J. J. J.; Martin, N.; Newell, D. R.; Rigoreau, L.; Smith, G. C. M.; Stockley, M.; Veuger, S.; Hickson, I. *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, 4210.
14. Morris, J.; Wishka, D. G. *Synthesis-Stuttgart* **1994**, 43.
15. All new compounds exhibited spectral ($^1$H NMR, IR, UV) and analytical (elemental analysis and/or LC-MS) data fully consistent with the assigned structures.
16. A general procedure for the MPS of thiopyran-4-ones is as follows: A solution of **4e** or **4f** (20 mg, 0.057 mmol) in dioxane (1 mL) was sonicated under $N_2$ for 5 min, before addition to a reaction tube containing the appropriate arylboronic acid (0.0625 mmol) and $K_2CO_3$ (19 mg, 0.14 mmol). A solution of tetrakis(triphenylphosphine)palladium(0) (3 mg) in dioxane (0.3 mL) was added, and the reaction mixture was stirred under $N_2$ at 90 °C for 18 h. After cooling, the product was isolated by chromatography on silica (isolute Si 500 mg cartridge), eluting with $CH_2Cl_2$:MeOH (3:1, 3 mL). Further purification by preparative HPLC furnished the required thiopyranones in 5–60% yield.
17. The DNA-PK used for in vitro assays was purified from HeLa cell nuclear extract. The known ability of DNA-PK to phosphorylate the serine-15 residue of a p53 peptide in vitro was exploited in a classic ELISA style assay using an antibody that only recognises the p53 serine-15 site when phosphorylated (Cell Signalling Technology). The primary antibody to p53 phosphoserine-15 was detected using an HRP conjugated goat anti-rabbit antibody (Pierce) with ECL reagent (NEN) being used for the readout. The ability of compounds to inhibit this phosphorylation event was monitored over a concentration range with $IC_{50}$ values generated from these results. Full details of the assay will be published elsewhere.