Bioorganic & Medicinal Chemistry 22 (2014) 756–761

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and biological evaluation of nitric oxide-donating analogues of sulindac for prostate cancer treatment

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ARTICLE INFO

Article history:
Received 22 September 2013
Revised 2 December 2013
Accepted 6 December 2013
Available online 12 December 2013

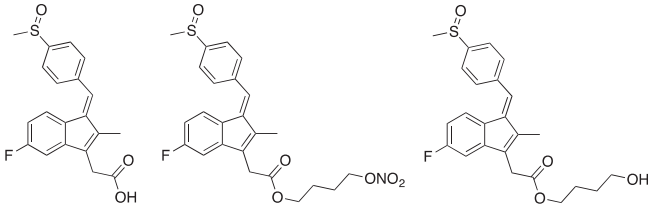
Keywords:
Nitric oxide
Sulindac analogues
Furoxan
Sydnonimine
Prostate cancer

ABSTRACT

A series of analogues of the non-steroidal anti-inflammatory drug (NSAID) sulindac **1** were synthesised tethered to nitric oxide (NO) donating functional groups. Sulindac shows antiproliferative effects against immortal PC3 cell lines. It was previously demonstrated that the effect can be enhanced when tethered to NO releasing groups such as nitrate esters, furoxans and sydnonimines. To explore this approach further, a total of fifty-six sulindac–NO analogues were prepared and they were evaluated as NO-releasing cytotoxic agents against prostate cancer (PCa) cell lines. Compounds **1k** and **1n** exhibited significant cytotoxic with IC_{50} values of 6.1 ± 4.1 and 12.1 ± 3.2 μM, respectively, coupled with observed nitric oxide release.

1. Introduction

In the United Kingdom, prostate cancer (PCa) accounts for 13% of male cancer deaths.[1] In 2010, 40,975 men were newly diagnosed with prostate cancer, accounting for almost 1 in 4 of all new male cancers diagnosed.[1] Both localised and locally advanced prostate cancers are highly responsive to androgen ablation therapies[2] including androgen receptor antagonists for example, bicalutamide and flutamide, GnRH agonists for example, leuprolide and more recently GnRH antagonists for example, degarelix. But over the course of 2–5 years most tumours relapse into a castration-resistant stage[2] that is subsequently treated with chemotherapy. Recently, research has been focused on the role of hypoxia in the induction of castration-resistant PCa.[3] Hypoxia has been shown to correlate with increased metastasis, angiogenesis and resistance to therapy.[3] In PCa, hypoxia is associated with poor patient prognosis.[4] It was demonstrated by Stewart et al. that an NO-donating analogue of sulindac **1**, NCX-1102 **2** has a pro-apoptotic and anti-invasive effect on PC3 prostate cancer cells.[5] In addition, the *des*-nitrato analogue NCX-112 **3**, had increased activity over sulindac **1**, but decreased compared to NCX-1102 **2**. The activity of NCX-1102 **2** was conserved under normoxic and hypoxic conditions. The master regulator of oxygen homeostasis in the cell is the transcription factor hypoxia-inducible factor 1 (HIF-1). Under hypoxic conditions HIF-1α expression was down-regulated upon treatment with NCX-1102 **2**; normal expression was observed when treated with sulindac **1** and NCX-112 **3**.[5] NCX-1102 **2** is predicted to function as a nitric oxide (NO) donor, and as such, the observed increase in activity was attributed to 'NO bioactivity'. In addition to decreased HIF-1α expression, a reduction in Akt phosphorylation was observed suggesting that the PI3K–Akt–mTOR signal transduction pathway is one of the mechanisms operating in the activity of NCX-1102 **2**, reinforcing earlier reports of interactions between this pathway and HIF-1α expression under hypoxic conditions.[6–8] Herein, we report the synthesis of a library of novel sulindac analogues exploring the relationship between sulindac **1** and a range of NO-donating functional groups. Including the identification of a series of furoxans with significant anti-proliferative activity at 50 μM.



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http://dx.doi.org/10.1016/j.bmc.2013.12.014

2. Results and discussion

2.1. Chemistry

We identified three classes of nitric oxide-donating functionalities to develop into sulindac-NO hybrids; nitrate esters, furoxans and sydnonimines. Based on literature examples where the oxidation state of sulfur in sulindac conferred differing biological activities in vitro,[9,10] we first began by preparing the known compounds sulindac sulfide **4** and sulindac sulfone **5**. To this effect, commercially available sulindac **1** (sulfoxide) was reduced to the corresponding sulfide **4** with $TiCl_4$/Zn, and oxidised to the sulfone **6** with Oxone® (Scheme 1).[11] With these starting materials in hand we turned our attention to a series of nitrate ester hybrids.

2.1.1. Nitrate esters

Nitrate ester alcohols **6**–**10** were readily prepared by halide substitution with silver nitrate. Ring-opening of THF **11** with NaI and TBDMSCl gave TBDMS protected iodobutanol **12**, followed by nitrate substitution and subsequent deprotection, provided the butyl linked nitrate ester alcohol **6** (Scheme 2),[12,13] analogous to NCX-1102 **2**. Further nitrate ester alcohols **7**–**10** were prepared from the available bromoalcohols **13**–**16** in good yields (Scheme 2).[14–16]

Ester formation of these nitrooxyalcohols **6**–**10** with sulindac congeners **1**, **4** and **5** using EDCI·HCl and catalytic DMAP generated the desired esters **1a**–**e**, **4a**–**e** and **5a**–**e** in good yield (Scheme 3). Appropriate hydroxybutyl ester control compounds were also prepared, analogous to NCX-112 **3**. Ester formation with mono-TBDSMS 1,4-butanediol followed by deprotection with camphorsulfonic acid furnished the required alcohols **1g**, **4g** and **5g** (Scheme 4).

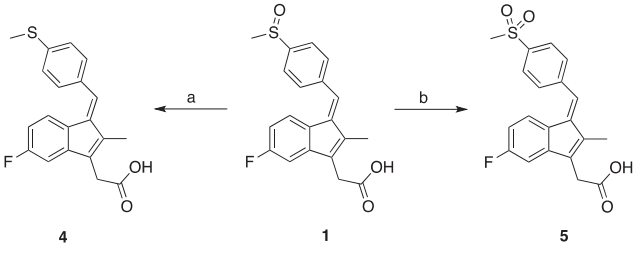
2.1.2. Furoxans

Furoxan alcohols **19a**–**i** were readily prepared from bis(phenylsulfonyl)furoxan **18**.[17,18] Treatment of phenyl(sulfonyl)acetic acid **17** with refluxing HNO_3/AcOH, generated the desired furoxan by nitrile oxide dimerisation (Scheme 5).[17]

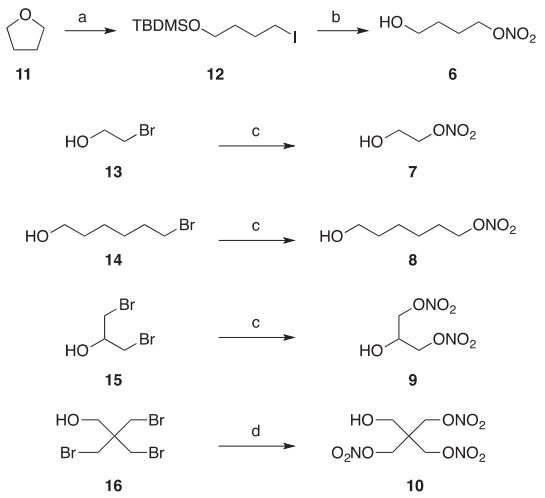
Selective substitution at the 4-position of furoxan **18** was accomplished by treatment with the corresponding diol and 50% w/w NaOH (Scheme 5).[17] The preparation of hydroxyethyl-furoxan **19a** and hydroxyethyl(ethoxy)-furoxan **19e** required mono-TBDMS protection of the diol to avoid side products. TBDMS deprotection with camphorsulfonic acid provided the furoxans **19a** and **19e**. Esterification of furoxans **19a**–**i** with acids **1**, **4** and **5** using the previously established EDCI conditions provided esters **1h**–**p**, **4h**–**p** and **5h**–**p** (Scheme 6).

2.1.3. Sydnonimines

3-Phenylsydnonimine **21** was prepared from aniline **20** based on the modified procedure reported earlier.[19] Alkylation to the aminoacetonitrile and subsequent N-nitrosation and acid catalysed ring closure furnished the required sydnonimine **21** (Scheme 7).[19]



Scheme 1. Synthesis of sulindac sulfide **4** and sulindac sulfone **6**. Reagents and conditions: (a) $TiCl_4$, Zn, THF, rt, 0.5 h, quantitative; (b) Oxone®, 1:1 MeOH, H_2O, rt, 1 h, quantitative.



Scheme 2. Synthesis of nitrate ester alcohols **6**–**10**. Reagents and conditions: (a) TBDMSCl, NaI, THF, 55 °C, 18 h, quant.; (b) (i) $AgNO_3$, CH_3CN, −10 °C to rt, 1 h, (ii) H_2O, 1 h, 29%; (c) (a) $AgNO_3$, CH_3CN, reflux, 6 h 85–90%; (d.) $AgNO_3$, CH_3CN, rt, 7 d, 26%.

Acylation of congeners **1**, **4** and **5** with **21** using EDCI·HCl and DMAP provided the sydnonimine amides **1q**, **4q** and **5q** (Scheme 8). The use of acetonitrile as a cosolvent was required to aid dissolution of **21** in the reaction. Acylation of **21** with *p*-nitrophenylchloroformate provided activated sydnonimine **22** for attachment to linkers (Scheme 7).[19] To this effect, activation of carboxylic acids **1**, **4** and **5** with carbonyl diimidazole and the addition of an excess of ethanolamine or ethylenediamine allowed for selective monoacylation to provide alcohols **1r**, **4r**, **5r** and amines **1s**, **4s** and **5s** (Scheme 9). Reaction of alcohols **1r**, **4r**, **5r** and amines **1s**, **4s** and **5s** with activated carbamate **22** in acetonitrile at 90 °C provided the respective carbamates **1t**, **4t**, **5t** and ureas **1u**, **4u** and **5u** (Scheme 9).

2.2. Biological results

2.2.1. Cytotoxicity results

PC3 cells (hormone insensitive human prostate cancer cells) obtained from the European Collection of Cell Cultures (ECACC), Salisbury, UK were used to determine cytotoxicity of the sulindac analogues. The cells were cultured in RPMI 1640 with 5% FCS and seeded in 96 well plates at a concentration of approximately 3000 cells/well. Sulindac-NO analogues were prepared in dimethyl sulfoxide (DMSO) at a final DMSO concentration of 0.05% in the culture media. Initially, the sulindac analogues were incubated at 50 μM with the cells for 72 h and cell growth measured at 24 h intervals using the crystal violet method.[20] The inhibitory effect on cell growth was recorded as a percentage of cells remaining compared to medium control (100% cell viability) (Table 1 and Supplementary information, Tables 1–3).

As with sulindac (sulfoxide) **1**, the corresponding sulfide **4** and sulfone **5** had no effect on PC3 cells at 50 μM (Table 1). In addition, the 4-hydroxybutyl esters **1g**, **4g** and **5g** showed no significant cytotoxicity (Supplementary information, Table 1). 4-Nitrooxybutyl esters **1a**, **4a** and **5a** demonstrated a strongly cytotoxic effect at 50 μM, with **4a** showing improved activity over NCX-1102 (Table 1). Further development of the nitrate ester series to investigate increased linker length, and the number of nitrate esters per unit of sulindac, provided a series of compounds which had no cytotoxic activity, but with two exceptions (Supplementary information, Table 2). These were 2-nitrooxyethyl ester **1b** and dinitrate ester **1d**. They displayed an overall reduction in cell