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Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

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To cite this article: Mahesh M. Savant, Kartik D. Ladva & Archna B. Pandit (2018) Facile synthesis of highly functionalized novel pyrazolopyridones using oxoketene dithioacetal and their anti-HIV activity, Synthetic Communications, 48:13, 1640-1648, DOI: 10.1080/00397911.2018.1458239

To link to this article: https://doi.org/10.1080/00397911.2018.1458239

SYNTHETIC COMMUNICATIONS®
2018, VOL. 48, NO. 13, 1640–1648
https://doi.org/10.1080/00397911.2018.1458239

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Facile synthesis of highly functionalized novel pyrazolopyridones using oxoketene dithioacetal and their anti-HIV activity

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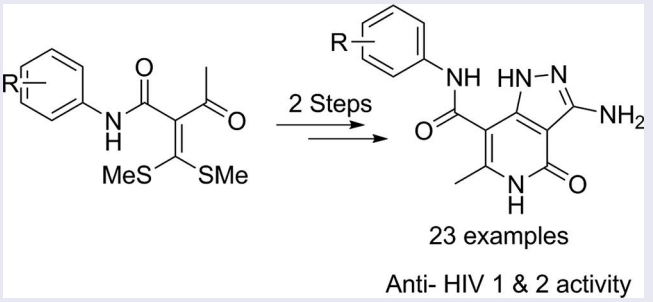
ABSTRACT

A series of novel 3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-aryl-1*H*-pyrazolo[4,3-c]pyridine-7-carboxamide have been synthesized starting from various oxoketene dithioacetals. The cyclocondensation reaction of 2-(bis(methylthio)methylene)-3-oxo-*N*-arylbutanamide **2a–w** with cyanoacetamide using NaO*i*Pr as base under reflux condition afforded novel highly functionalized pyridone **3a–w** derivatives. Further, [3 + 2] cyclocondensation reaction of pyridones with hydrazine in the presence of alcohol was yielded pyrazolopyridones (23 nos) **4a–w** with excellent yields. All newly synthesized compounds were evaluated for *in vitro* anti-HIV activity using MTT method. Most of these compounds have showed moderate to potent activity against HIV-1 (III_B) and HIV-2 (ROD) strains with an IC_{50} ranging from >18 IC_{50} [μg/ml] to <100 IC_{50}[μg/ml]. Among them, compounds **4j** and **4v** were identified as the most promising compound for both types of HIV strains. (IC_{50} = 18 μg/ml). Three compounds **4l, 4m**, and **4p** have been found potent anti-HIV 1 and 2 activity against MT-4 cells.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 29 November 2017

KEYWORDS
Anti-HIV activity; highly functionalized pyrazolopyridone; ketene dithioacetals; MTT method

Introduction

The human immunodeficiency virus (HIV)—the etiologic agent of acquired immunodeficiency syndrome (AIDS)—is the fastest growing cause of death in women of reproductive age.[1] The current therapy against the HIV type 1 (HIV-1), which is the etiological agent of AIDS, is based on six of categorized drugs: nucleoside/nucleotide reverse transcriptase

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inhibitors (NRTIs/NtRTIs); nonnucleoside reverse transcriptase inhibitors (NNRTIs); protease inhibitors (PIs); cell entry inhibitors [fusion inhibitors (FIs); coreceptor inhibitors (CRIs)]; and integrase inhibitors (INIs).[2] In 2003, NNRTIs like lersiverine, bearing pyrazole moiety, have received great attention in the field of anti-HIV research.[3] Up to now, several polyazaaromatics have been studied and developed as NNRTI, including pyridones and pyrazoles.[4–6]

Condensed polyazaaromatics have attracted significant attention from the scientific community because of their relevance in medicinal chemistry. Polyazaaromatics frequently can be observed in numerous bioactive small molecules, and in particular, fused with various heterocycles, such as pyrazole, pyridine, and pyrimidine, have been used as key pharmacophores.[7] As shown in Figure 1, a blockbuster drug, sildenafil citrate (**1**),[8] and a potent anticancer agent (**2**)[9] contain polyazaaromatics fused with privileged heterocycles as core skeletons. In addition, 1*H*-pyrazolo[3,4-b]pyridine **3** is recognized as a privileged substructure, inhibitors were discovered by optimizing a fragment screening hit scaffold using structure guided design. These inhibitors show potent Gram-positive antibacterial activity and low resistance incidence against clinically important pathogens.[10] Structure-based design of novel inhibitors that interact with the K103 backbone of HIV-reverse transcriptase has led to the discovery of NNRTIs that contain an unusual 1*H*-pyrazolo[3,4-c]pyridazine heterocycles (**4**).[11] Recently, pyrazolopyridone **5** found as a novel class of noncovalent DprE1 inhibitor with potent antimycobacterial activity.[12] Moreover, novel 2-pyridone derivatives **6** have been synthesized and found potent anti-HIV-1 (III_B) at low micromolar concentrations ($EC_{50} = 0.15–0.84\ \mu M$), comparable to that of Nevirapine and Delavidine.[13] These examples emphasize the importance

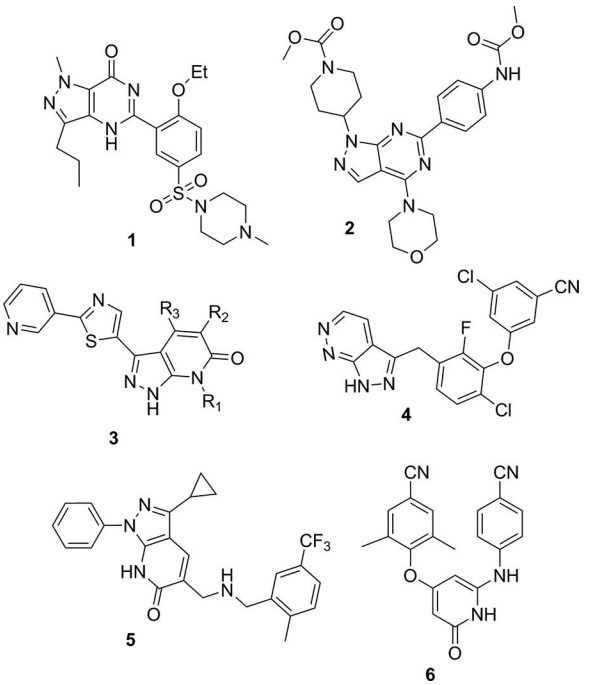


Figure 1. Biologically active pyrazolopyridones.

of pyridone, pyrazolopyridone as well as pyrazolopyridines, as key pharmacophores in bioactive small molecules. Several diverse biological activities have been reported for condensed pyrazolopyridone ring systems such as MAP kinase,[14] antimicrobial,[15] anti-Leishmania,[16] PDE inhibitors,[17] anti-HIV,[18] anticancer.[19] Literature survey reveals the significance of highly substituted pyrazolopyridone in medicinal chemistry. Herein, we report an efficient and easy synthesis of novel compounds based on the pyrazolopyridone backbone scaffold bearing a carboxamide side chain using various oxoketene dithioacetals for biological interest.

Fused pyrazolopyridone ring systems are present in a variety of biologically active compounds (both naturally occurring and synthetic). Although a large number of methods for their synthesis have been documented in the literature, many of them require multistep procedures using intermediates which are not readily available.[20–34]

Results and discussion

In our efforts to discover novel anti-HIV agents[2] and synthesis of various heterocyclic compounds using oxoketene dithioacetals,[35–36] we have observed that oxoketene dithioacetals are versatile intermediate for the synthesis of pyrazolopyridone derivatives. All the synthesized compounds were screened for their *in vitro* anti-HIV activity against human HIV cell line. These compounds were required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method.

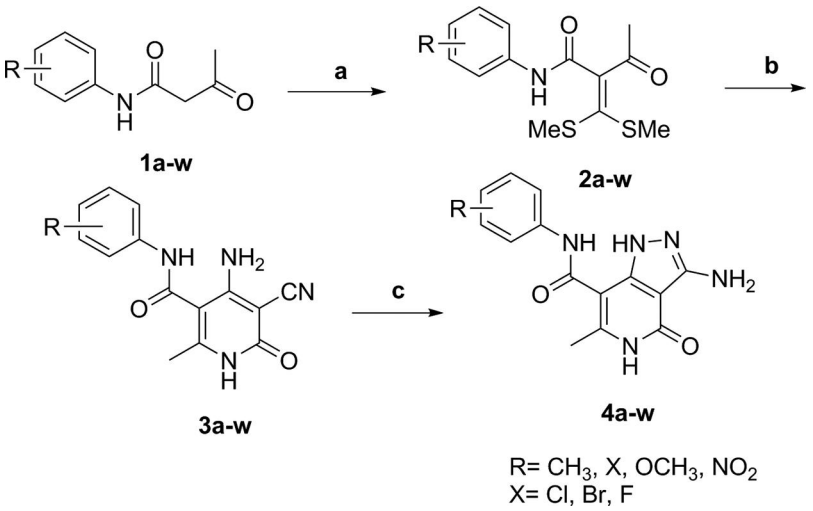
Initially, the reaction of 2-(bis(methylthio)methylene)-3-oxo-*N*-phenylbutanamide (**2a**) with cyanoacetamide (CA) was performed using sodium methoxide in methanol. The reaction of **2a** with CA in sodium methoxide was afforded the product **3a** in 75% yield with long reaction time (Table 1). To optimize the reaction condition for the synthesis of compound **3a**, various sodium alkoxides were utilized in respective alcohol. As a result, we found the reaction of **2a** with CA was faster and afforded the pyridone **3a** in good yield in the presence of sodium isopropoxide and isopropyl alcohol. Various oxoketene dithioacetals **2a–w** have been prepared by our reported method.[36] Moreover, the cyclocondensation reaction of pyridones with 4-methylbenzenesulfonhydrazide was not promising to yield pyridones. We have observed that separation of pyridones salt was required to furnish the product in high yield. The one postreaction of oxoketene dithioacetals, cyanoacetamide, and hydrazine hydrate was not clean and yielded the desired product.

The resulting pyridones **3a–w** was further reacted with hydrazine hydrate in isopropyl alcohol under reflux condition to afford the desired pyrazolopyridone derivatives in excellent yield with short reaction time (Scheme 1). The synthesized compounds were confirmed by IR, Mass, ^{1}H, and ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their *in vitro* antiviral activity using HIV-1 (III_B) and HIV-2 (ROD) strains

In mechanism, the cyanoacetamide on the treatment with base generates an anion at active methylene group which attacks on β-carbon of oxoketene dithioacetal. The amine

Table 1. Optimization reaction of **2a** with cyanoacetamide using various bases.

Entry	Base	Time h	Yield %
1	NaOMe	7	75
2	NaOEt	6	82
3	NaO*i*Pr	4	88



Scheme 1. Synthesis of highly functionalized pyrazolopyridone **4a–w** starting from oxoketene dithioacetals **2a–w**. Reagents and conditions: (a) i: K_2CO_3, CS_2, DMF, 0-5 oC to rt 2 hr, ii: MeI, DMF, 0-5 oC to rt overnight (b) CA, NaOiPr, iPA, rt to reflux, 4 hr (c) NH_2NH_2, iPA, Reflux.

nucleophile attacks on carbonyl carbon and forms sodium salt of pyridine moiety by removal of methylthio and water molecule. The sodium salt on acidification afforded pyridone. The binucleophile hydrazine hydrate on reaction with pyridone forms pyrazolopyridone (Scheme 2).

In vitro anti-HIV assay of pyrazolopyridones

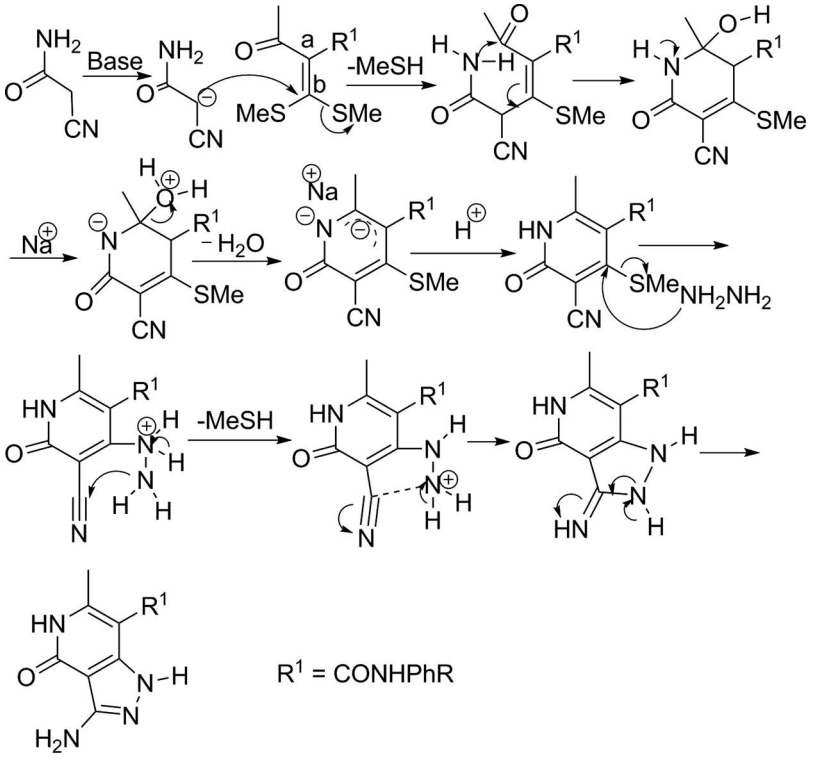
Three groups of the compounds were studied for anti-HIV activities according to the IC_{50} values measured on the wildtype HIV-1 & two cell lines: strong anti-HIV effect $0 < IC_{50} < 20$ μg/ml (two compounds: **4j** and **4v**), moderate anti-HIV effect $20 < IC_{50} < 50$ μg/ml (compounds **4l, 4m**, and **4p** Table 2), and no antiproliferative effect, where $IC_{50} > 50$ μg/ml was found (Table 2). The lowest IC_{50} value for HIV-1 and HIV-2 strain was found <19.4 and <18.4 μg/ml, respectively (**4j**), while some compounds were ineffective even at the applied 100 μg/ml concentration (i.e., **4b, 4e, 4h**, and **4t**)

Structure–activity reveals that electronegative substitution increases the potency, thus chloro and fluoro groups (**4j, 4l**) play a vital role. Also, methyl substitution at *ortho* and *meta* positions (**4v**) shows moderate biological potential. Bulky substitution at phenyl group does not favorable and this explains the inactivity of compounds having nitro in **4i, 4t**, and **4w** and methoxy in **4e** and **4k** molecules.

Experimental

Materials and methods

Melting points were determined on an electrothermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was performed on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365 nm) or with iodine vapor. IR spectra were recorded on a Bruker alpha FTIR spectrophotometer



Scheme 2. Plausible reaction mechanism for the formation of pyrazolopyridone.

using DRS prob. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE II (400 MHz, 100 MHz) spectrometer in DMSO. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were recorded using a direct inlet probe on an Agilent technology GCMS5977A mass spectrometer. All reagents were purchased from Fluka, Loba, and Rankem and used without further purification. Anti-HIV screening has been done using protocol reported in the literature.[37]

General procedure for the synthesis of pyridones 3a–w

To a well stirred mixture of cyanoacetamide (10 mmol) and sodium isopropoxide (10 mmol) in isopropyl alcohol was added the solution of ketene dithioacetals **2a–w** (10 mmol) in isopropyl alcohol within 10–15 min. The resulting reaction mixture was further stirred at rt for 15 min and heated to reflux for 4 to 5 hr on water bath. The reaction was being monitored by TLC Hexane: EtOAc (7:3). After completion of the reaction, the solvent was evaporated under *vacuuo* and the resulting solid was treated with dilute HCl solution. Thus, the obtained solid was filtered, washed with water, and dried at rt to afford analytically pure products. The solid products were used for next step without further purification.

General procedure for the synthesis of pyrazolopyridones 4a–w

The mixture of substituted pyridones **3a–w** (5 mmol) and hydrazine hydrate (10 mmol) in isopropyl alcohol was refluxed for appropriate time on water bath. After completion of the

Table 2. Physical and in vitro anti-HIV activity data of highly functionalized pyrazolopyridones **4a–w**.

				HIV-1 III_B		HIV-2 ROD	
Entry	R	Time min	Yield %	IC_{50}[a]	CC_{50}[b]	IC_{50}[a]	CC_{50}[b]
4a	Ph	50	96	94.70	≥94.70	94.70	≥94.70
4b	4-CH_3	65	95	100.00	>100.00	100.00	>100.00
4c	4-OCH_3	40	96	88.8	=88.8	73.8	=73.8
4d	4-F	45	97	89	=89	83.9	=83.9
4e	2-OCH_3	55	88	100.00	>100.00	100.00	>100.00
4f	2-CH_3	65	89	83.3	=83.3	96.1	=96.1
4g	4-Cl	60	91	92.6	=92.6	93.5	=93.5
4h	4-Et	45	93	98.00	>98.00	98.00	>98.00
4i	4-NO_2	50	95	56.8	=56.8	71.5	=71.5
4j	3-Cl,4-F	60	85	19.4	=19.4	18.4	=18.4
4k	5-Cl, 2-OCH_3	65	87	73.1	=73.1	54.8	=54.8
4l	2,5-diCl	70	88	49.1	=49.1	48.8	=48.8
4m	2,5-diCH_3	60	89	45.6	=45.6	49.5	=49.5
4n	4-Cl, 2-CH_3	70	83	68.6	=68.6	60.4	=60.4
4o	3,4-diF	75	92	99.03	99.03	97.1	=97.1
4p	2-Cl	75	84	48.7	=48.7	42.6	=42.6
4q	2-F	60	85	98.9	=98.9	82.9	=82.9
4r	4-Br	55	92	94.6	=94.6	108.20	108.20
4s	3,4-diCl	60	94	71.08	71.08	62.9	=62.9
4t	3-NO_2	55	84	101.00	≥101.00	101.00	≥101.00
4u	3-CH_3	65	88	67.2	=67.2	68.3	=68.3
4v	2,3-diCH_3	65	89	19.8	=19.8	19	=19
4w	2-OCH_3, 4-NO_2	70	91	86.7	=86.7	81.1	=81.1
AZT				7.6	7.6	7.8	7.8

[a]The IC_{50} values are the concentrations of the compounds which inhibit tumor cell growth by 50%, [b]Compd. Concd. (μg/ml) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the colorimetric MTT method.

reaction, solid product was appeared in the reaction. The reaction mixture was cooled to room temperature. The separated product was then filtered and washed with *i*PA and dried at room temperature to furnished analytically pure products.

Conclusion

In summary, we have described an efficient and easy synthesis of highly functionalized novel pyrazolopyridone derivatives in excellent yields. The reaction of various oxoketene dithioacetals with cyanoacetamide was afforded the pyridone derivatives with good yields in the presence of base. Synthesis of pyridone was examined using various bases and sodium isopropoxide was found as an efficient base. The pyridones were further reacted with hydrazine hydrate to furnish pyrazolopyridones. The significance of current method is excellent yields of novel pyrazolopyridones with short reaction time for biological interest. All compounds were screened against HIV-1 III_B and HIV-2 ROD strains. Among them, **4j** and **4v** compounds were found active against both HIV-1 and 2 cell having IC_{50} ranging from <19.4, <19.8, and <18.4, <19 μg/ml, respectively. Anticancer screening of synthesized pyrazolopyridone derivatives is under investigation.

Funding

Authors are thankful for financial assistance given under UGC-MiRP, New Delhi, Sanction No.: 47-605/13(WRO); Dated: 20/05/2014 and Shree M. & N. Virani Science College, Rajkot for providing central instrumentation & laboratory facilities and Prof. Pannecouque Rega Institute for Medical Research, KU Leuven, Belgium for providing Anti-HIV analysis.

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