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Short communication

Synthesis and antigenotoxic activity of some naphtho[2,1-*b*]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives

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Abstract

In this study, some pyranotriazolopyrimidine derivatives were synthesised by reacting ethoxymethyleneamino derivatives with hydrazides. Their structures were elucidated by IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data and elemental analyses. Antigenotoxic activity of the obtained compounds was tested in *Escherichia coli* PQ37 by using the SOS chromotest.

Keywords: Iminoethers; Triazolopyrimidines; SOS chromotest; Antigenotoxic activity

1. Introduction

Much attention has been paid to the synthesis of various triazolopyrimidines due to their antibacterial [1–4], anti-inflammatory [5–7] and herbicidal activities [8–11]. With this in mind and in continuation of our previous works[12,13] on the synthesis of new fused 4*H*-pyrans using iminoethers **1** as starting material we report here the synthesis of a variety of pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **2** along with their antigenotoxic activity.

2. Chemistry

Iminoethers are known to react with compounds containing $-NH_2$ moiety such as hydrazides [14–16]. In fact iminoethers **1** possess two reactive sites, a cyano group and an imidic carbon. These groups render them susceptible to react with hydrazides under toluene reflux in the presence of few drops of acetic acid to give new compounds. As is shown in Scheme 1, two plausible pathways and different products could be expected.

(i) Successive two nucleophilic additions of $-NH_2$ group on the imidic carbon and on the cyano function to yield amidopyranopyrimidines **2′**. In this case hydrazides react with iminoethers **1** like hydroxylamine, primary amines [11] and tosylhydrazine [12]. The intermediate **2′** can be evolved by an intracyclisation via elimination of water to give pyranotriazolopyrimidines **2**.

(ii) Successive two nucleophilic additions of two nitrogen atoms of NH_2-NH- moiety on the reactive sites of iminoethers **1** to yield pyranotriazepines **2″** or their isomers **2‴**. The IR spectrum revealed the lack of the characteristic absorption bands corresponding to cyano, amino and C=O groups. The ^{1}H NMR spectrum points out the absence of ethoxy group, and showed the presence of two characteristics signals at 6 and 9 ppm assigned, respectively, to the 14*H* and $-CH{=}N-$ protons. Based on these data, it is clear that the reaction was proceeded to produce naphtho[2,1-*b*]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **2** rather than pyranotriazepines **2″** or **2‴**.

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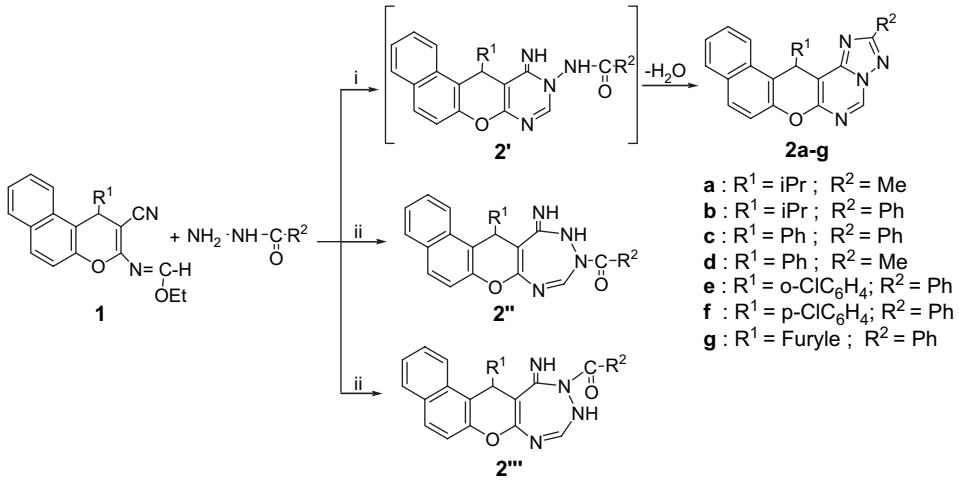


Scheme 1.

3. SOS chromotest assay

SOS chromotest assay was performed to verify the presence or absence of genotoxic effect of the tested triazolopyrimidine derivatives **2a**–**g**. The same test allows us to look for a possible antigenotoxic activity of same compounds by incubating *Escherichia coli* PQ 37 strain in the presence of both extract and mutagen (nifuroxazide). The complete genotype, as well as *E. coli* PQ 37 strain construction details are described by Quillardet and Hofnung [17].

4. Results and discussion

Different quantities (2, 10 and 50 μg/assay) of the tested chemical compounds were dissolved in dimethylsulfoxide and added to the bacterial culture. Each concentration was tested in triplicate. As a measure of genotoxicity and antigenotoxicity, the SOSIP (SOS-induction potency) was calculated from the linear part of the induction factor–dose-response curve. The induction factor (IF) was calculated as the ratio of R_c/R_0, where R_c is equal to β-gal activity/AP activity determined for the compound at concentration c and R_0 is equal to β-gal activity/AP activity in the absence of the test compound. The β-gal and AP activities were calculated according to the method described by Quillardet and Hofnung [17]. Antigenotoxicity was expressed as a percentage of inhibition of genotoxicity induced by nifuroxazide according to the formula: (%) inhibition $= 100 - (IF_1 - IF_0/IF_2 - IF_0) \times 100$, where IF_1 is the induction factor in the presence of the test compound, IF_2 is the induction factor in the absence of the compound and IF_0 is the induction factor of the negative control in the absence of nifuroxazide and the tested compound.

In a series of experiments preceding the antigenotoxicity studies, it was ascertained that the different amounts of the tested compounds added to the indicator bacteria does not influence its viability. Likewise, induction factor values obtained when the tested compounds are incubated with *E. coli* PQ37 do not change significantly, when compared to that of non-treated bacteria (IFs close to 1) (Table 1).

As shown in Table 2, all chemical compounds were effective in reducing the IF induced by nifuroxazide (5 μg/assay), a directly acting genotoxic (IF = 12.88 ± 0.05). Unfortunately, no correlation between substituents and antigenotoxic activity of the compounds was found. Compounds **2a**, **2c** and **2g** were more effective antigenotoxic molecules than the triazolopyrimidines **2b**, **2d**, **2e** and **2f**. Compounds **2a**, **2c** and **2g** significantly decrease the SOSIP of nifuroxazide by 99%, at the concentrations of 2, 50 and 10 μg/assay, respectively.

However, compounds **2b**, **2d**, **2e** and **2f** showed a weak antigenotoxic activity when respectively 50, 2, 2 and 50 μg/assay of each compound were added to the assay system.

Table 1
Study of genotoxicity of different naphthopyranotriazolopyrimidines using the "SOS chromotest" with *E. coli* PQ37

Test compound	Dose (μg/assay)	IF
PC	5	12.88
NC	0	1
2a	50	1.39
	10	1.10
	2	1.01
2b	50	1.25
	10	1.19
	2	1.11
2c	50	1.40
	10	1.24
	2	1.02
2d	50	1.56
	10	1.32
	2	1.12
2e	50	1.62
	10	1.21
	2	1.16
2f	50	1.67
	10	1.33
	2	1.44
2g	50	1.30
	10	1.40
	2	1.30

IF: Induction factor; PC: positive control, bacteria treated with nifuroxazide; NC: negative control, non-treated bacteria.

Table 2
Effect of different naphthopyranotriazolopyrimidines on genotoxicity induced by nifuroxazide (5 μg/assay) in the "SOS chromotest" using *E. coli* PQ37

Test compound	Dose (μg/assay)	Inhibition of genotoxicity (%)
Nifuroxazide	5	–
0	0	0
2a	2	99
	10	98
	50	95
2b	2	62
	10	61
	50	63
2c	2	78
	10	86
	50	99
2d	2	70
	10	61
	50	56
2e	2	64
	10	60
	50	48
2f	2	32
	10	38
	50	48
2g	2	99
	10	99
	50	75

Positive control: bacteria treated with nifuroxazide; negative control: non-treated bacteria (0).

The SOSIP of nifuroxazide decreased by 63%, 70%, 64% and 48%, respectively.

Antigenotoxic activity of the molecules **2a**, **2d**, **2e** and **2g** is dose-independent; whereas that of molecules **2b**, **2c** and **2f** is dose-dependant.

5. Conclusion

The triazolopyrimidine derivatives are very well known compounds since many years, but very little is known about their antigenotoxic activity. This research examined the antigenotoxic activity of new triazolopyrimidines **2a–g** prepared by the reaction of iminoethers **1** with hydrazides. The antigenotoxic activity of compounds was carried out in *E. coli* PQ 37 strain. The biological behaviour revealed that there is no correlation between substituents and antigenotoxic activity of the compounds. However, the compounds **2a**, **2c** and **2g** were found to be more active than the other molecules.

6. Experimental protocols

6.1. Chemistry

NMR: NMR spectra were recorded on a Bruker AC 200 (^{1}H: 300 MHz, ^{13}C: 75 MHz) in $CDCl_3$ or DMSO-d_6 solution. All chemical shifts are recorded in parts per million (ppm) downfield from tetramethylsilane. Coupling constants ($^3J_{HH}$) are given in hertz (Hz).

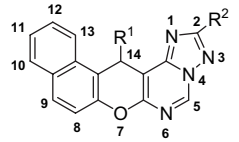
IR: IR spectra were determined for KBr discs on a JASCO FT-IR-420 spectrometer with the incertitude as $\pm 2\ cm^{-1}$ in the field of $4000-400\ cm^{-1}$.

Melting point: the melting points were determined in Electrothermal 9100 apparatus and are uncorrected.

The reactions were monitored by thin layer chromatography using aluminium sheets with silica gel 60 F_{254} (Merck).

6.2. Synthesis of triazolopyrimidines *2a–g*

A mixture of iminoether **1** (0.01 mmol) and hydrazide (0.01 mmol) in toluene (20 ml) was refluxed for 5 h in the presence of few drops of acetic acid. The solvent was evaporated to dryness under reduced pressure. The solid was collected by filtration. The crude product was purified by recrystallization from ethanol to give compounds **2a–g**.



2a-g

6.2.1. 14-Isopropyl-2-methyl-14H-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (*2a*)

Yield: 85%; m.p.: 190 °C; IR: νC=N: 1619 cm^{-1}; ^{1}H NMR ($CDCl_3$): 0.73 (d, $^3J_{HH} = 7.1$ Hz, 3H), 1.07 (d, $^3J_{HH} = 7.1$ Hz, 3H), 2.38 (m, 1H), 2.65 (s, 3H), 5.27 (d, $^3J_{HH} = 6.9$ Hz, 1H), 7.27–8.17 (m, 6H), 9.07 (s, 1H); ^{13}C NMR ($CDCl_3$): 15.13 (CH_3), 18.47 ((CH_3)$_2$CH), 21.72 ((CH_3)$_2$CH), 36.72 (C-14), 37.57 ((CH_3)$_2$CH), 101.69 (C-14a), 116.95–155.03 (C_{arom}), 157.27 (C-5), 167.49 (C-6a). Anal. Calc. for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.61; H, 5.39; N, 16.88.

6.2.2. 14-Isopropyl-2-phenyl-14H-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (*2b*)

Yield: 80%; m.p.: 289 °C; IR: νC=N: 1629 cm^{-1}; ^{1}H NMR ($CDCl_3$): 0.75 (d, $^3J_{HH} = 7.1$ Hz, 3H), 1.18 (d, $^3J_{HH} = 7.1$ Hz, 3H), 2.44 (m, 1H), 5.39 (d, $^3J_{HH} = 6.9$ Hz, 1H), 7.16–8.36 (m, 11H), 9.18 (s, 1H); ^{13}C NMR ($CDCl_3$): 17.93 ((CH_3)$_2$CH), 21.74 ((CH_3)$_2$CH), 36.43 (C-14), 37.36 ((CH_3)$_2$CH), 102.69 (C-14a), 116.74–154.24 (C_{arom}), 156.87 (C-5), 166.39 (C-6a). Anal. Calc. for $C_{25}H_{20}N_4O$: C, 76.51; H, 5.14; N, 14.28. Found: C, 76.41; H, 5.30; N, 14.30.

6.2.3. 2,14-Diphenyl-14H-naphtho[2,1-b]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (*2c*)

Yield: 67%; m.p.: 246 °C; IR: νC=N: 1632 cm^{-1}; ^{1}H NMR (DMSO): 6.46 (s, 1H), 7.13–8.35 (m, 16H), 9.62 (s, 1H); ^{13}C NMR (DMSO): 37.46 (C-14), 102.81 (C-14a), 115.25–153.34 (C_{arom}), 153.52 (C-5), 165.91 (C-6a). Anal. Calc. for $C_{28}H_{18}N_4O$: C, 78.86; H, 4.25; N, 13.14. Found: C, 78.71; H, 4.30; N, 13.11.

*6.2.4. 2-Methyl-14-phenyl-14H-naphtho[2,1-b]pyrano [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**2d**)*

Yield: 70%; m.p.: 248 °C; IR: νC=N: 1633 cm^{-1}; ^1H NMR ($CDCl_3$): 2.61 (s, 3H), 6.32 (s, 1H), 7.06–7.96 (m, 11H), 8.97 (s, 1H); ^{13}C NMR ($CDCl_3$): 14.73 (CH_3), 37.60 (C-14), 103.05 (C-14a), 115.06–153.24 (C_{arom}), 153.78 (C-5), 167.15 (C-6a). Anal. Calc. for $C_{23}H_{16}N_4O$: C, 75.81; H, 4.43; N, 15.38. Found: C, 75.61; H, 4.33; N, 15.14.

*6.2.5. 14-(2-Chlorophenyl)-2-phenyl-14H-naphtho[2,1-b] pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**2e**)*

Yield: 78%; m.p.: 306 °C; IR: νC=N: 1629 cm^{-1}; ^1H NMR ($CDCl_3$): 6.83 (s, 1H), 7.01–8.35 (m, 15H), 9.11 (s, 1H); ^{13}C NMR ($CDCl_3$): 35.04 (C-14), 99.95 (C-14a), 117.76–153.64 (C_{arom}), 153.82 (C-5), 165.81 (C-6a). Anal. Calc. for $C_{28}H_{17}ClN_4O$: C, 72.96; H, 3.72; N, 12.16. Found: C, 72.83; H, 3.63; N, 12.17.

*6.2.6. 14-(4-Chlorophenyl)-2-phenyl-14H-naphtho[2,1-b] pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**2f**)*

Yield: 82%; m.p.: 274 °C; IR: νC=N: 1633 cm^{-1}; ^1H NMR (DMSO): 6.41 (s, 1H), 7.26–8.28 (m, 15H), 9.63 (s, 1H); ^{13}C NMR (DMSO): 37.03 (C-14), 102.38 (C-14a), 118.08–153.46 (C_{arom}), 154.62 (C-5), 166.07 (C-6a). Anal. Calc. for $C_{28}H_{17}ClN_4O$: C, 72.96; H, 3.72; N, 12.16. Found: C, 72.88; H, 3.73; N, 12.24.

*6.2.7. 14-Furan-2-yl-2-phenyl-14H-naphtho[2,1-b]pyrano [3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**2g**)*

Yield: 80%; m.p.: 273 °C; IR: νC=N: 1636 cm^{-1}; ^1H NMR ($CDCl_3$): 5.22 (s, 1H), 6.12–8.28 (m, 14H), 9.08 (s, 1H); ^{13}C NMR ($CDCl_3$): 30.50 (C-14), 99.47 (C-14a), 106.49–153.46 (C_{arom}), 153.55 (C-5), 166.27 (C-6a). Anal. Calc. for $C_{26}H_{16}N_4O$: C, 74.99; H, 3.87; N, 13.45. Found: C, 74.88; H, 3.83; N, 13.43.

6.3. Assessment of antigenotoxic activity

A sample of 0.1 ml of an overnight culture of the strain to be tested is diluted in 5 ml of Luria Broth (L) medium supplemented with ampicillin (10 μg/ml) and the culture is incubated at 37 °C in a gyratory incubator up to 2×10^8 bacteria/ml. This takes about 2 h. Then, 1 ml of the culture is diluted in 9 ml of fresh L medium. Fractions of 0.6 ml are distributed into a series of disposable-stoppered glass test containing 20 μl of samples of the compounds to be tested when we looked for the genotoxic effect; 10 μL of samples of the compounds to be tested and 10 μl of nifuroxazide (5 μg/ml) when we looked for antigenotoxic activity.

The mixtures are incubated with shaking for 2 h at 37 °C. After incubation, 0.3 ml fractions are withdrawn from each tube and put into a new series of tubes. There are thus two series of tubes each containing 0.3 ml fractions. One series will be used for the β-galactosidase assay and the other series for the alkaline phosphatase assay. The two enzyme assays can be performed in parallel.

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