Synthesis and biological evaluation of new indazole derivatives

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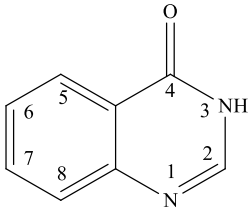
Abstract
New N-methyl and N-ethyl substitutions in the indazole nucleus are reported by reacting 3-(2-aminobenzamido)indazole and the appropriate trimethyl/triethyl orthobenzoate. Single crystal X-ray analysis confirms the N-ethylation position for the 3-(1-ethyl-1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one derivative **3f**. Compounds **11a-d** and **3a-d** were tested to evaluate their antimicrobial, antiproliferative and COX inhibitory activities, showing scarce or moderately antiproliferative activity and some inhibitory activity against COX-1 and COX-2.

Keywords: N-Methyl/N-ethyl alkylation, 4(3*H*)-quinazolinone, indazole, crystallography, biological activity

Introduction

The 4(3*H*)-quinazolinone ring system **I** represents a very attractive scaffold to obtain new molecules endowed with a wide range of pharmacological properties.



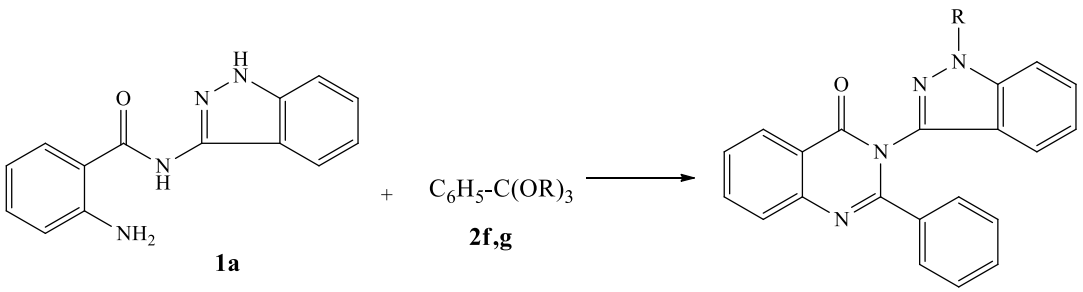
It has been reported that the 4(3*H*)-quinazolinone moiety is present in a large number of compounds with anticonvulsant and CNS depressant,[1] analgesic and anti-inflammatory,[2] antitumoral,[3] antileukemic,[4] and antifungal[5] activities. As these pharmacological properties

mostly depend on the substituents at 2 and 3 positions, we were interested since a long time to study the influence of different substituents on the 4(3*H*)-quinazolinone ring. Recently, the synthesis and the biological evaluation of some 2-phenyl-3-(substituted-benzothiazol-2-yl)-4(3*H*)-quinazolinone derivatives, endowed with anti-inflammatory and antimicrobial activities, have been reported.[6] In particular, the interesting antiproliferative activity of 3-indazolyl-substituted 4(3*H*)-quinazolinones bearing hydrogen, methyl or ethyl moieties at C(2) have drawn our attention.[7,8] These observations prompted us to study in depth the 3-indazolyl-substituted 4(3*H*)-quinazolinones through the synthesis of new derivatives bearing a phenyl group at C(2), in order to ascertain the role and the possible advantage of this substitution over the hydrogen, methyl or ethyl- analogs regarding the above mentioned biological activities.

Results and Discussion

Chemistry

The synthesis of the 3-indazolyl-substituted 4(3*H*)-quinazolinones **3a** was first carried out by refluxing the 3-(2-aminobenzamido)indazole **1a** in triethyl orthobenzoate **2f** according to the previously described procedure (Scheme 1).[9]

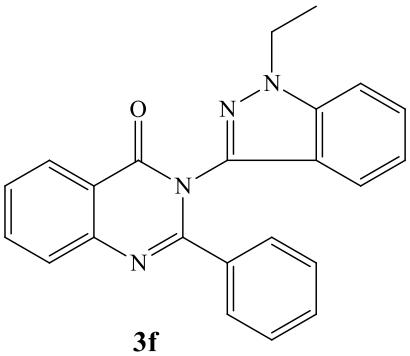


	a	f	g
R	H	C_2H_5	CH_3

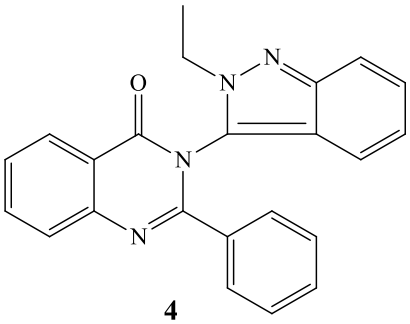
Scheme 1

Surprisingly, instead of the expected 3-(1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one **3a**, a product having mass at M+28 was isolated. The analytical and the spectroscopic data of this product suggested an N-ethylation reaction of the indazole nucleus. In principle this reaction could give both the 3-(1-ethyl-1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one derivative **3f** and the 3-(2-ethyl-2*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one isomer **4**. For a conclusive elucidation of the structure, an X-ray diffraction analysis was performed (see crystallography).

Furthermore, the reaction of compound **1a** with trimethyl orthobenzoate **2g** gave the *N*-methylated indazole nucleus, probably the **3g** derivative, as with the ethyl analogue.



3f



4

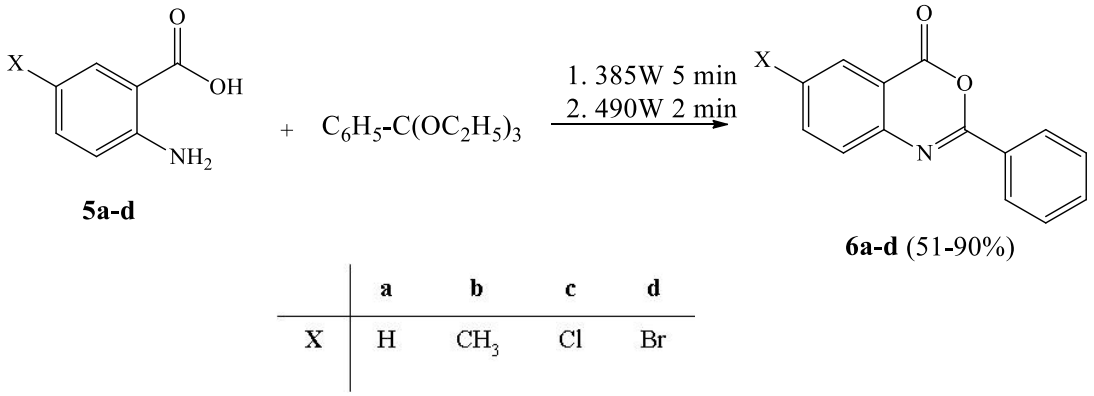
A review of the literature showed that orthoesters are potential alkylating agents.[10] Our findings represent the first example of methylation/ethylation in the indazole series, evidencing that the methylation/ethylation of indazole nucleus is deeply influenced by the orthoester used in the reaction. In fact, the methylation/ethylation was observed only with the orthobenzoates, while the unethylated 3-indazolyl-4(3*H*)-quinazolinones were obtained if triethyl orthoformate, triethyl orthoacetate or triethyl orthopropionate were used.[8] The possible reason of such result was likely due to the higher boiling points of orthobenzoates with respect to the other orthoesters (Table 1).

Table 1. Boiling Points (°C) of some orthoesters

Orthobenzoates	B.P. °C
Triethyl orthoformate	143
Triethyl orthoacetate	142
Triethyl orthopropionate	155-160
Triethyl orthobenzoate	239-241
Trimethyl orthobenzoate	224

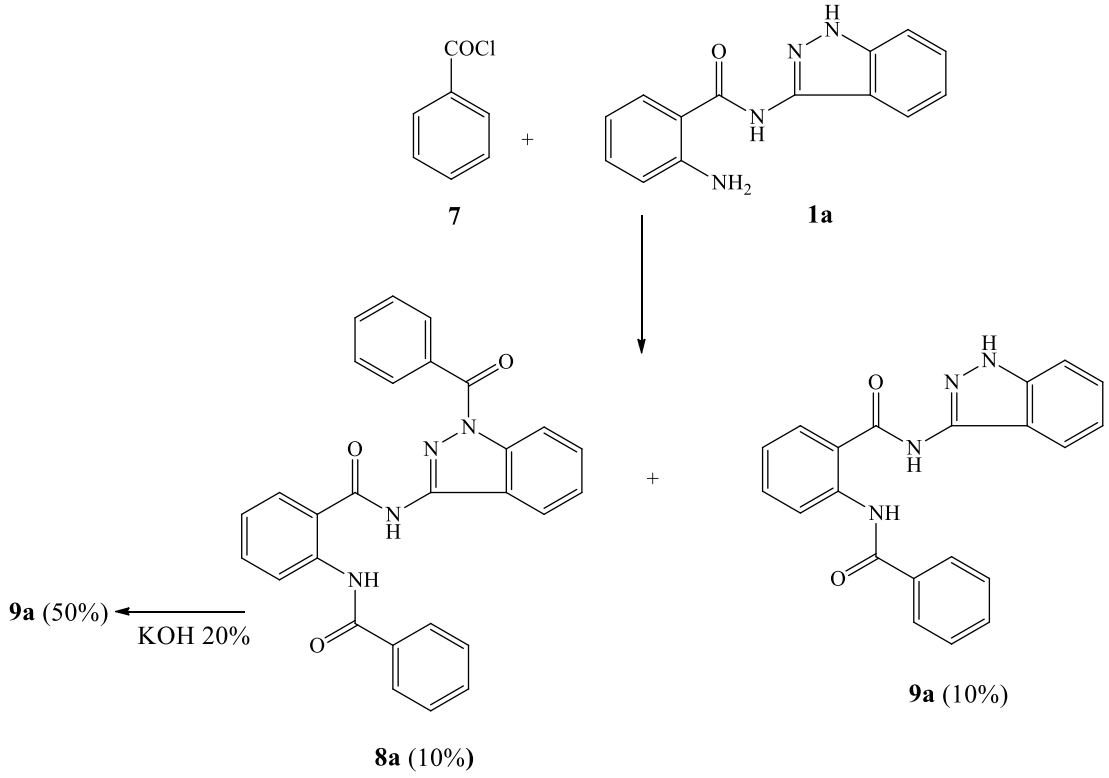
In order to verify this statement, a mixture of 3-(2-aminobenzamido)indazole **1a** in triethyl orthobenzoate **2f** was left at 160°C for 5 hours (Scheme 1) giving the 3-(1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one **3a** as the main product (20% yield) together with the starting material, instead of the N-ethyl derivative **3f** (Scheme 1).

For the synthesis of the novel 2-phenyl-3-(substituted-indazol-3-yl)-4(3*H*)-quinazolinones **3**, we decided to evaluate two of the most frequently employed strategies: (i) *via* the benzoxazinone **6** as intermediate and ii) *via* the *N*-(indazol-3-yl)-2-benzamidobenzamide **9.** The starting materials, 2-phenyl-3,1-benzoxazin-4-ones **6a-d**[12-15] were obtained with a novel procedure starting from the appropriate 2-aminobenzoic acid **5a-d** and the triethyl orthobenzoate **2f** by microwave assisted synthesis (Scheme 2). *N*-(Indazol-3-yl)-2-aminobenzamide **1a**,[9] and 3-aminoindazole **10,**[11] were prepared according to the previously described method.



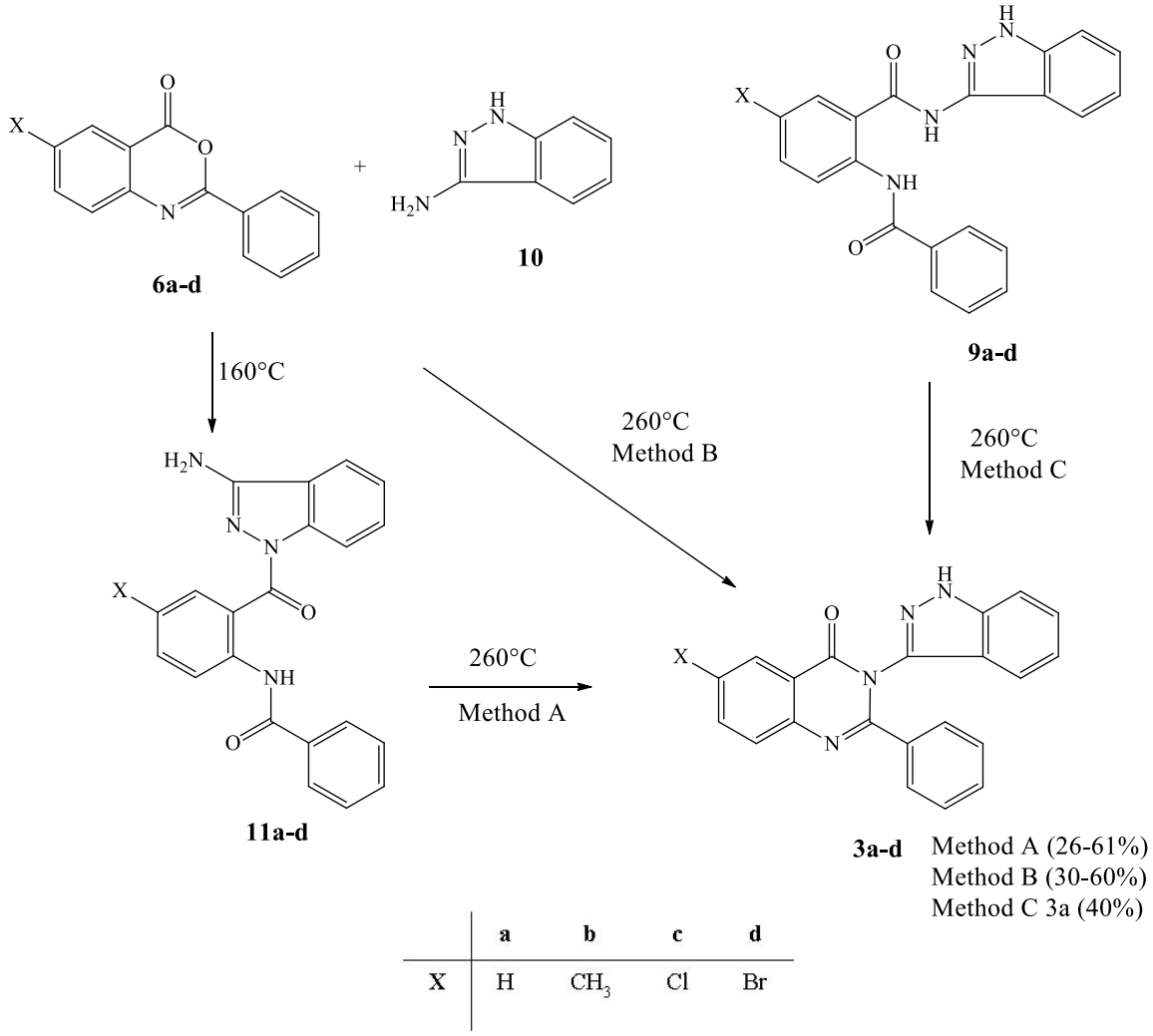
Scheme 2

Compound **9a** was prepared by amidation of the *N*-(indazol-3-yl)-2-aminobenzamide **1a** with benzoyl chloride **7** in pyridine. This reaction gave a mixture from which two products were isolated and identified as **8a** and **9a** (Scheme 3). By stirring **8a** at room temperature in 20% alcoholic sodium hydroxide solution (24 h), the *N*-(indazol-3-yl)-2-benzamidobenzamide **9a** was obtained Moreover, by maintaining the crude mixture at room temperature in 20% alcoholic sodium hydroxide solution (24 h), only the desired **9a** was directly obtained in 50% yield.



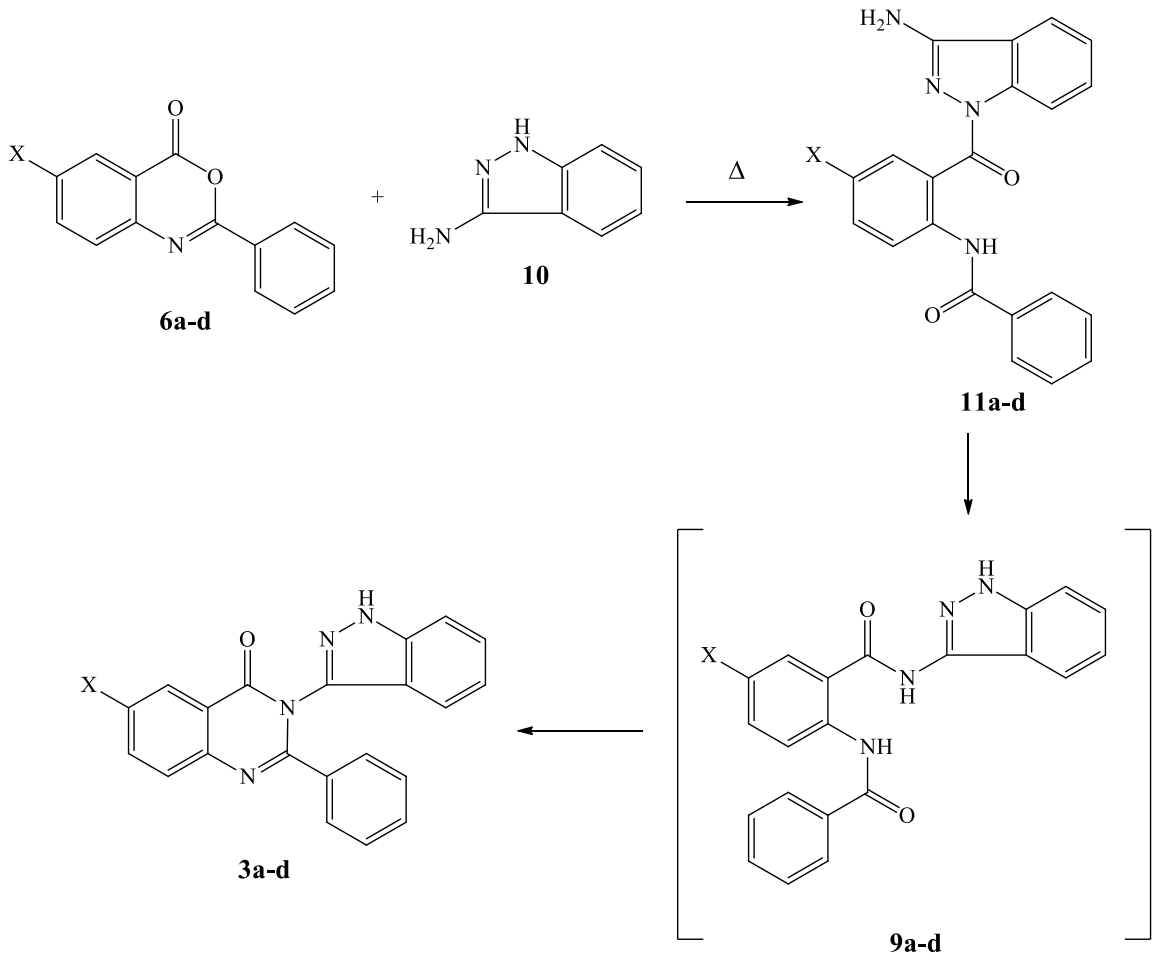
Scheme 3

For that concerning the procedure having the benzoxazinone **6** as intermediate, the *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides **11a-d** were obtained *via* the fusion of the appropriate benzoxazinone **6a-d** with 3-aminoindazole **10** at 160 °C for 15 minutes. Then the *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides **11a-d** were heated at 260 °C for 15 minutes to give the desired 2-phenyl-3(indazol-3-yl)-quinazolin-4(3*H*)-ones **3a-d** that were also obtained in one step by fusion of benzoxazinones **6a-d** with 3-aminoindazole **10** at 260 °C for 15 minutes (Scheme 4).



Scheme 4

On the basis of the results of these two synthetic routes we can conclude that the cyclization of **11a-d** into **3a-d** occurred *via* thermal rearrangement of the benzoylamidobenzoyl group through the possible intermediate **9a-d** (Scheme 5). A similar rearrangement was previously reported in the pyrazole series.[16]



	a	b	c	d
X	H	CH_3	Cl	Br

Scheme 5

Moreover, according to the second procedure, the 2-phenyl-3-(indazol-3-yl)-quinazolin-4(3*H*)-one **3a** was also obtained by heating the *N*-(indazol-3-yl)-2-benzamidobenzamide **9a** at 260°C for 15 minutes (Scheme 4). Compound **3a** corresponds in all aspects to the same compound obtained by the alternative routes shown in Schemes 1 and 4.

The structures of the new compounds **3a-d**, **8a**, **9a** and **11a-d**, were characterized by analytical and spectroscopic measurements (see Experimental).

Crystallography

The X-ray analysis allows a conclusive attribution of the structure **3f**, showing the N-ethylation position of the indazole nucleus, as reported in Figure 1.

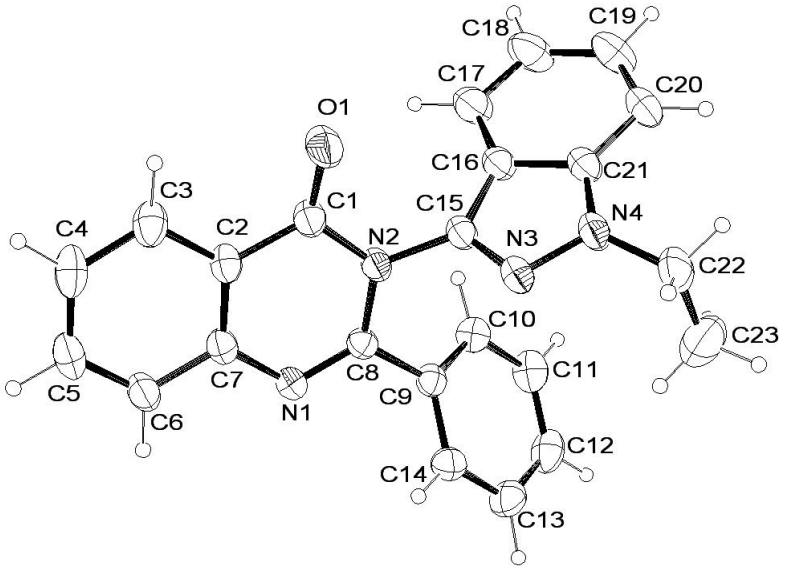


Figure 1. ORTEP[17] of **3f** showing the atom-numbering scheme, as used for 3-(indazol-3-yl)-methyl-quinazolin-4(3*H*)-one[9] (ellipsoids are at the 50% probability).

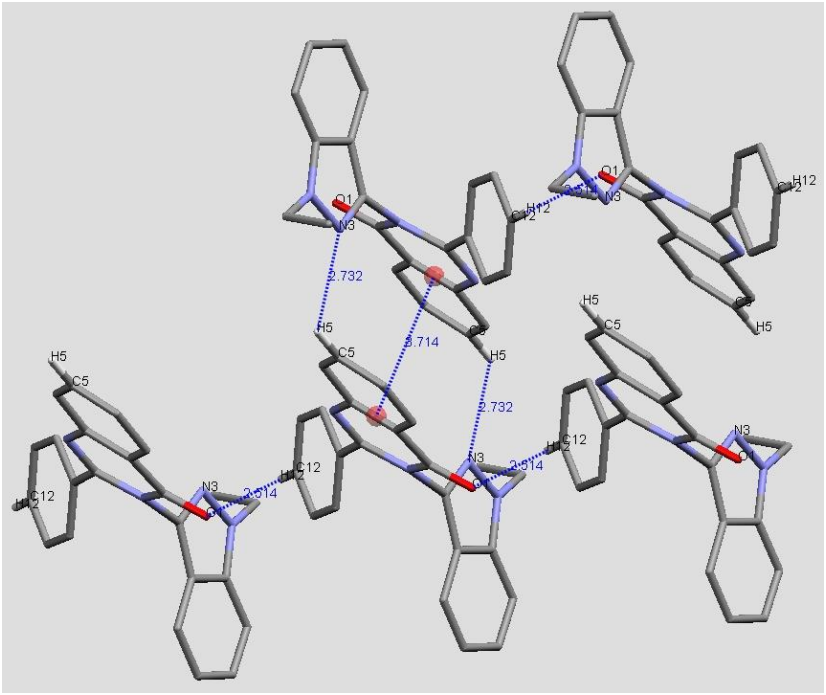


Figure 2. Intermolecular interactions of **3f** (H-bonds and centroids distances are reported).

The molecular structure is characterized by the bicyclic quinazolinone moiety, substituted at C(8) with a phenyl ring, connected to an indazole substituted at N(4) by an ethyl group. The dihedral angle between the two bicyclic systems is 69.8(1)°, with the N(3)-C(15)-N(2)-C(1)

torsion angle of -63.7(1)°. The benzene is inclined of 45.7(1)° and 60.1(1)° with respect to the quinazolinone and indazole systems respectively.

A comparison of the present structure with that of the 3-(indazol-3-yl)-methyl-quinazolin-4(3*H*)-one[9] shows as the insertion of the phenyl at C(8) causes a reduction of 16.7(1)° in the torsion angle N(3)-C(15)-N(2)-C(1). The substitution of the hydrogen with the ethyl group modifies as expected the intermolecular interactions for that regarding the O(1), which weakly interacts with C(12)^{I}-H at a distance of 2.52(1)Å, angle 151(1)° (^{I} at x+1, y+1, z). Additional contacts concern N(3)…C(5)^{II}-H at 2.73(1)Å, angle 132(1)° (^{II} at 2-x, -y, -z). Molecular stacking is also present between the quinazolinone moieties centrosymmetrically related (Figure 2).

Biology

Compounds **3a-d** and **11a-d** were preliminary tested to evaluate their antimicrobial effects against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. fecalis* and *C. albicans* as well as their antiproliferative activity against K-562 (human chronic myelogenus leukemia), HT29 (colon cancer cells) and NCI-H460 (human large cell lung cancer cells). The analysis of the biological data shows no antimicrobial activity at 50 μM concentration in all the synthesized compounds. Results of the antiproliferative activity exhibit scarcely or moderately activity ranging from 10.0-20.0 % (10 μM) against K562, 12.9-52.3 % (100 μM) against HT29, and 13.1-41.7 % (100 μM) against NCIH460 cell lines. Compounds **3a-d** and **11a-d** were also tested as COX1/COX2 inhibitors (Table 2).

Table 2. IC_{50} values (μM) on COX-1 and COX-2 for compounds **3a-d** and **11a-d**

Compound	COX-1	COX-2	SI
3a	89.3	102.0	0.87
3b	127.1	35.4	3.59
3c	88.5	34.7	2.55
3d	69.7	66.4	1.05
11a	80.0	72.7	1.1
11b	ns	113.0	0.079
11c	43.8	114.3	0.38
11d	132.2	208.0	0.64
NS398 [0.2 μM]	14.1	73.2	0.19

ns = not significant (% inhibition < 10%); SI = Selectivity index (IC50COX-1/ IC50COX-2).

Data reported in Table 2 show that compound **3** and **11** are endowed of inhibitory activity against COX-1 and COX-2. In particular, the *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides **3a**, **11b-d** seem to be lightly selective towards the COX-1 isoform, while the 3-(indazol-3-yl)-quinazolin-4(3*H*)-one derivatives **3b-d 11a** possess a moderate COX-2 selectivity.

Conclusions

Reactions of 3-(2-aminobenzamido)indazole and the appropriate trimethyl/triethyl orthobenzoate give N-methyl/N-ethylation of the indazole nucleus, representing the first example of *N*-alkylation in the indazole series. This is probably related to the higher boiling point of the orthobenzoate than the other orthoesters, for which the *N*-alkylation does not occur. The correct position of the *N*-alkylation has been clarified by the X-ray analysis of the 3-(1-ethyl-1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-one derivative **3f**. The 2-phenyl-3(indazol-3-yl)-quinazolin-4(3*H*)-ones **3a-d** and *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides **11a-d** present scarcely or moderately antiproliferative activity against K-562 (human chronic myelogenus leukemia), HT29 (colon cancer cells) and NCI-H460 (human large cell lung cancer cells). In vitro testing for **3** and **11** indicated that they are endowed with inhibitory activity against COX-1 and COX-2. Further investigation of biology of this kind of products is in progress and the results were published elsewhere.

Experimental Section

General. All melting points were determined on a Buchi 530 capillary melting points apparatus; IR spectra were recorded with a Jasco Spectrum IR-810. System spectrophometer as solid in KBr disc or nujol mull supported on NaCl disks; ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d_6 at 300.13 and 75.47 MHz respectively, using a Bruker AC series 300 MHz spectrometer (TMS as internal reference). Mass spectra were recorded on a JEOL jms-0I-SG-2 spectrometer at 75 eV (100µA). Microanalyses (C, H, N), performed in the Redox snc laboratories, were within ±0.4 % of the theoretical values. The microwave reactions were carried out in an Anton Paar Synthos 3000 instrument.

Reaction of benzoyl chloride (7) with 2-amino-*N*-(1*H*-indazol-3-yl)benzamide (1a)

Method A. To a stirred cold (ice bath, 0-5 °C) solution of 2-amino-*N*-(1*H*-indazol-3-yl)benzamide **1a** (10 mmol) in pyridine (8 ml), benzoyl chloride **7** (10 mmol) was added dropwise. Stirring was continued for 24 h then the solution was concentrated to dryness under reduced pressure. The resulting solid was flash chromatographed[18]: silica gel (230-400 mesh), external column diameter 3.0 cm, petroleum ether-diethylether 7:3 as eluent, each fraction 50 ml. The fractions 6-8 were collected and evaporated under reduced pressure to give 2-benzamido-*N*-(1-benzoyl-1*H*-indazol-3-yl)benzamide **8a** which was, in turn, crystallized from ethanol. The fractions 31-50 were collected and evaporated under reduced pressure to give *N*-(indazol-3-yl)-2-benzamidobenzamide **9a** which was, in turn, crystallized from ethanol. **8a:** Yield 10 %; mp 220°C; IR (KBr) cm^{-1} 3270 (broad NH), 1646-1680(3xCO); ^{1}H-NMR(δ) DMSO-d_6 7.29-9.46(18H, a set of signals, 2xC_6H_5 and 2x C_6H_4), 11.61-11.49 (2H, broad, exchangeable with D_2O, 2xNH); ^{13}C-NMR(δ) DMSO-d_6 115.22 (CH_{Ar}), 121.08 (C_{Ar}), 121.38 (CH_{Ar}), 122.36 (C_{Ar}),

123.00 (CH_{Ar}), 123.18 (CH_{Ar}), 124.40 (CH_{Ar}), 127.03 ($2XCH_{Ar}$), 127.84 ($2XCH_{Ar}$), 128.64 ($2XCH_{Ar}$), 129.66 (CH_{Ar}), 129.87 (CH_{Ar}), 130.48 ($2xCH_{Ar}$), 131.82 (CH_{Ar}), 131.93 (CH_{Ar}), 132.48 (CH_{Ar}), 133.09 (C_{Ar}), 134.36 (C_{Ar}), 138.87 (C_{Ar}), 140.33 (C_{Ar}), 145.72 (C_{Ar}), 164.62 (CO), 167.13 (CO), 168.32 (CO). Anal. Calcd. For $C_{28}H_{20}N_4O_3$: C, 73.03; H, 4.38; N, 12.17; Found: C, 73.00; H, 4.41; N, 11.97%. **9a:** Yield 10%; mp 260°C; IR (KBr) cm^{-1} 3170 (NH), 1647 (CO); ^{1}H-NMR(δ) DMSO-d_6 7.18-8.74 (13H, a set of signals, $2xC_6H_4$ and C_6H_5), 11.18 (1H, s, exchangeable with D_2O, NH), 12.21 (1H,s,exchangeable with D_2O, NH), 13.01 (1H, s, exchangeable with D_2O, NH); ^{13}C-NMR(δ) DMSO-d_6 110.37 (CH_{Ar}), 117.15 (C_{Ar}), 119.93 (CH_{Ar}), 120.68 (C_{Ar}), 120.88 (CH_{Ar}), 121.28 (CH_{Ar}), 123.13 (CH_{Ar}), 126.42 (CH_{Ar}), 126.93 ($2XCH_{Ar}$), 128.85 ($2XCH_{Ar}$), 129.23 (CH_{Ar}), 132.00 (CH_{Ar}), 132.72 (CH_{Ar}), 134.38 (C_{Ar}), 139.30 (C_{Ar}), 139.43 (C_{Ar}), 141.03 (C_{Ar}), 164.53 (CO), 168.27 (CO). Anal. Calcd. For $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72; Found: C, 71.02; H, 4,72; N, 15.52%.

Method B. To a stirred cold (ice bath, 0-5 °C) solution of 2-amino-*N*-(1*H*-indazol-3-yl)benzamide **1a** (10 mmoles) in pyridine (8 ml), benzoyl chloride **7** (10 mmoles) was added dropwise. Stirring was continued for 24 h then the solution was concentrated to dryness under reduced pressure. The residue was left under magnetic stirrer for 24 h with 20% potassium hydroxide aqueous solution, the solid that separated out was filtered off and recrystallized from ethanol to give pure **9a** which was identical to that synthesized by method A (Rf, mp, mixed mp, IR, ^{1}HNMR); yields 50%.

General procedure for 2-phenyl-3,1-benzoxazin-4-ones (6a-d)[12-15]

10 mmol of the appropriate 2-aminobenzoic acid **5a-d** were added of 4.5 ml of triethyl orthobenzoate. The microwave assisted reactions were run for 7 min at 385 W for the first 5 min then at 490 W for the last 2 min. The crude white product was filtered and crystallized from ethyl acetate to give pure **6a-d**; yields 51-90%.

General procedure for *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides (11a-d)

Equimolar amounts of the appropriate 2-phenyl-benzoxazin-4-one **6a-d** and 3-aminoindazole **10**[9] were heated at 160° C for 15 minutes. After this time, the solid obtained was crystallized from ethanol to give pure **11a-d**.

11a. Yield 37%; mp 167°C (ethanol); IR (KBr) cm^{-1} 3456-3213 (NH, NH_2), 1672 (CO); ^{1}H-NMR (δ) DMSO-d_6 6.57 (2H ,s ,NH_2 exchangeable with D_2O), 7.30-8.34 (13H, a set of signals, C_6H_5, C_6H_4, C_6H_4), 10,37 (1H, s, NH exchangeable with D_2O); ^{13}C-NMR(δ) DMSO-d_6 115.57 (CH_{Ar}), 120.24 (C_{Ar}), 120.69 (CH_{Ar}), 123.84 (CH_{Ar}), 123.93 (CH_{Ar}), 124.00 (CH_{Ar}), 127.30 ($2XCH_{Ar}$), 128.20 (C_{Ar}), 128.40 ($2XCH_{Ar}$), 129.68 (CH_{Ar}), 130.66 (CH_{Ar}), 130.81 (CH_{Ar}), 131.65 (CH_{Ar}), 134.41 (C_{Ar}), 135.96 (C_{Ar}), 140.06 (C_{Ar}), 153.34 (C_{Ar}), 165.01 (CO), 165.75 (CO). Anal. Calcd. For $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4,53; N, 15,72; Found: C, 70.52; H, 4,30; N, 15.87%.

11b. Yield 10%; mp 110°C (ethanol); IR (KBr) cm^{-1} 3453-3289 (NH,NH_2), 1678 (CO). ^{1}H-NMR(δ) DMSO-d_6 2.35 (3H,s,CH_3), 6.59 (2H, s, NH_2 exchangeable with D_2O) 7.34-8.30 (12H,

a set of signals, C_6H_5, C_6H_4, C_6H_3), 10.25 (1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13; Found: C, 71.68; H, 5.03; N, 14.93%.

11c. Yield 30%; mp 203°C (ethanol); IR (KBr) cm^{-1} 3468-3221 (NH,NH_2), 1670 (CO); ^{1}H-NMR(δ) DMSO-d_6 6.61 (2H, s, NH_2 exchangeable with D_2O), 7.36-8.30 (12H, a set of signals, C_6H_5, C_6H_4, C_6H_3), 10.41(1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{21}H_{15}ClN_4O_2$: C, 64.54; H, 3.87; N, 14.34; Found: C, 64.86; H, 3.76; N, 14.53%. **11d:** Yield 25%; mp 200°C (ethanol); IR (KBr) cm^{-1} 3427-3218 (NH,NH_2), 1637 (CO); ^{1}H-NMR(δ) DMSO-d_6: 6.64 (2H, s, NH_2 exchangeable with D_2O), 7.39-8.32 (12H, a set of signals, C_6H_5, C_6H_4, C_6H_3), 10.44(1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{21}H_{15}BrN_4O_2$: C, 57.95; H, 3.47; N, 12.87; Found: C, 58.17; H, 3.23; N, 13.18%.

General procedure for 2-phenyl-3(indazol-3-yl)-quinazolin-4(3*H*)-ones (3a-d)

Method A. 0.5 mmol of the appropriate *N*-(2-(3-amino-1*H*-indazole-1-carbonyl)phenyl)benzamides **11a-d** were heated at 260° C for 15 minutes. After this time, the residue was crystallized from ethanol to give pure **3a-d**.

3a. Yield 61%; mp 226°C (ethanol); IR (KBr) cm^{-1} 3304 (NH), 1676 (CO); H-NMR(δ) DMSO-d_6 7.12-8.25 (13H, a set of signals, C_6H_5, C_6H_4, C_6H_4), 13.19 (1H, s, NH exchangeable with D_2O). ^{13}C-NMR(δ) DMSO-d_6 110.67 (CH_{Ar}), 119.31 (C_{Ar}), 119.38 (CH_{Ar}), 120.26 (C_{Ar}), 121.34 (CH_{Ar}), 126.54 (CH_{Ar}), 126.63 (CH_{Ar}), 127.51 (CH_{Ar}), 127.61 (3XCH_{Ar}), 128.09 (2XCH_{Ar}), 129.29 (CH_{Ar}), 135.11 (CH_{Ar}), 135.21 (CH_{Ar}), 138.85 (C_{Ar}), 140.82 (C_{Ar}), 147.15 (C_{Ar}), 155.35 (C_{Ar}), 161.57 (CO). Anal. Calcd. For $C_{21}H_{14}N_4O$: C, 74.54; H, 4.17; N, 16.56; Found: C, 74.41; H, 4.23; N, 16.65%.

3b. Yield 26%; mp 265°C (ethanol).); IR (KBr) cm^{-1} 3366 (NH), 1693 (CO); ^{1}H-NMR(δ) DMSO-d_6 2.50 (3H, s, CH_3), 7.11-8.06 (12H, a set of signals, C_6H_5, C_6H_4, C_6H_3), 13.16(1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{22}H_{16}N_4O$: C, 74.98; H, 4.58; N, 15.90; Found: C, 74.79; H, 4.47; N, 16.03%.

3c. Yield 33%; mp 260°C (ethanol).); IR (KBr) cm^{-1} 3303 (NH), 1687 (CO); ^{1}H-NMR(δ) DMSO-d_6 7.12-8.17 (12H, a set of signals, C_6H_5, C_6H_4, C_6H_3, 13.21 (1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{21}H_{13}ClN_4O$: C, 67.66; H, 3.51; N, 15.03; Found: C, 67.82; H, 3.71; N, 15.17%.

3d. Yield 50%; mp 260°C (ethanol).); IR (KBr) cm^{-1} 3304 (NH), 1688 (CO); ^{1}H-NMR(δ) DMSO-d_6 7.12-8.30 (12H, a set of signals, C_6H_5, C_6H_4, C_6H_3), 13.20 (1H, s, NH exchangeable with D_2O). Anal. Calcd. For $C_{21}H_{13}BrN_4O$: C, 60.45; H, 3.14; N, 13.43; Found: C, 60.64; H, 2.97; N, 13.20%.

Method B. Equimolar amounts of the appropriate 2-phenyl-benzoxazin-4-one **6a-d** and 3-aminoindazole **10** were heated at 260° C for 15 minutes. After this time, the solid obtained was crystallized from ethanol to give pure **3a-d** (yield 30-60%)**,** which were identical to that synthesized by method A (Rf, mp, mixed mp, IR, ^{1}HNMR).

Method C. . 0.5 mmol of *N*-(indazol-3-yl)-2-benzamidobenzamide **9a** was heated at 260° C for 15 minutes. After this time, the reaction mixture was crystallized from ethanol to give pure **3a**

which was identical to that synthesized by methods A and B (Rf, mp, mixed mp, IR, ^{1}HNMR); yields **3a** 40%.

General procedure for 3-(1-alkyl-1*H*-indazol-3-yl)-2-phenylquinazolin-4(3*H*)-ones (3f,g)

4 Mmoles of 3-(2-aminobenzamido)indazole **1** were dissolved by heating in 10 ml of orthobenzoate **2f,g** and the solution was refluxed for 10 h. Evaporation of the solvent under reduced pressure gave a residue which was recrystallized from ethanol to give pure **3g,f**.

3f. Yield 55-65 %; mp 208-210°C; MS (m/z) 366 (M^+); IR (KBr) cm^{-1} 1685 (CO); ^{1}H-NMR(δ) DMSO-d_6 1.01(2H, t, CH2), 4.28-4.33 (3H, broad, CH_3), 7.12-7.67 (13H, a set of signals, C_6H_5 and 2xC_6H_4); ^{13}C-NMR(δ) DMSO-d_6 14.71 (CH_3), 43.15 (CH_2), 109.98 (CH_{Ar}), 119.66 (CH_{Ar}), 119.85 (C_{Ar}), 120.30 (C_{Ar}), 121.37 (CH_{Ar}), 126.52 (CH_{Ar}), 126.54 (CH_{Ar}), 127.36 (2XCH_{Ar}), 127.53 (CH_{Ar}), 127.64 (CH_{Ar}), 128.09 (2XCH_{Ar}), 129.12 (CH_{Ar}), 135.05 (C_{Ar}), 135.20 (CH_{Ar}), 137.47 (C_{Ar}), 139.80 (C_{Ar}), 147.15 (C_{Ar}), 155.35 (C_{Ar}), 161.38 (CO). Anal. Calcd. For $C_{23}H_{18}N_4O$: C, 75.39; H, 4.95; N, 15.29; Found: C, 75.31; H, 4.64; N, 15.33%.

3g. Yield 60-65%; mp 168° C; MS (m/z) 352 (M^+); IR (KBr) cm^{-1} 1686 (CO); ^{1}H-NMR(δ) DMSO-d_6 3.92 (3H, s, CH_3), 7.15-7.96 (13 H, a set of signals, C_6H_5 and 2xC_6H_4). ^{13}C-NMR(δ) DMSO-d_6 35.54 (CH_3), 110.13 (CH_{Ar}), 119.48 (CH_{Ar}), 119.80 (C_{Ar}), 120.21 (C_{Ar}), 121.46 (CH_{Ar}), 126.52 (CH_{Ar}), 126.63 (CH_{Ar}), 127.46 (2XCH_{Ar}), 127.58 (CH_{Ar}), 127.62 (CH_{Ar}), 128.16 (2XCH_{Ar}), 129.35 (CH_{Ar}), 134.90 (C_{Ar}), 135.27 (CH_{Ar}), 137.21 (C_{Ar}), 140.63 (C_{Ar}), 147.09 (C_{Ar}), 155.22 (C_{Ar}), 161.55 (CO). Anal. Calcd. For $C_{22}H_{16}N_4O$: C, 74.98; H, 4.58; N, 15.90; Found: C, 74.59; H, 4.37; N, 16.94%.

Crystallography

Table 3. Crystal data and structure refinement for **3f**

Identification code	**3f**
Empirical formula	C_{23} H_{18} N_4 O_1
Formula weight	366.41
Temperature (K)	293(2)
λ Mo kα (Å)	0.71073
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions (Å,°)	a = 9.115(2) α = 111.07(3) b = 10.264(3) β = 93.84(3) c = 12.533(2) γ = 114.52(2)
Volume (Å^3)	963(3)
Z	2
Calculated density (Mg/m^3)	1.264
F(000)	384
Crystal size (mm)	0.8 x 0.7 x 0.6

Table 3. Continued

θ range for data collection (°)	3.33 to 28.00
Limiting indices	-12≤h≤12, -13≤k≤12, 0≤l≤16
Reflections collected / unique	4835 / 4629 [R(int) = 0.035]
Completeness	99.8 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4629 / 0 / 299
Goodness-of-fit	1.187
Final R indices [I>2σ(I)]	R1 = 0.0664, wR2 = 0.1566
R indices (all data)	R1 = 0.0800, wR2 = 0.1652

Crystals of **3f** were obtained as colorless prisms from a water-methanol 1:1 solution by slow evaporation at room temperature. They were mounted on an Enraf Nonius CAD-4 diffractometer using MoKα ($\lambda_{MoK\alpha}$=0.71073Å) radiation at room temperature (293K). The lattice parameters were determined by least-squares refinements of 25 high angle reflections. The structure was solved by direct methods,[19] and the refinement was carried out by full-matrix least-squares with SHELX-97.[20] All non-H-atoms were refined anisotropically. A summary of the crystal data, data collection, and structure refinement is presented in Table 3; selected bond lengths and angles are reported in Table 4. Geometrical calculations were carried out with the program PARST.[21]

Table 4. Selected bond lengths [Å] and angles [°] for **11f**

O(1)-C(1)	1.215(2)	O(1)-C(1)-N(2)	120.1(2)
N(1)-C(8)	1.289(2)	O(1)-C(1)-C(2)	125.7(2)
N(1)-C(7)	1.395(3)	N(1)-C(8)-N(2)	123.5(2)
N(2)-C(1)	1.404(2)	N(1)-C(7)-C(2)	122.3(2)
N(2)-C(8)	1.402(2)	N(2)-C(1)-C(2)	114.2(2)
N(2)-C(15)	1.430(2)	C(8)-N(1)-C(7)	118.2(2)
N(3)-C(15)	1.314(3)	C(8)-N(2)-C(1)	122.1(1)
N(3)-N(4)	1.362(2)	C(1)-C(2)-C(7)	119.5(2)
N(4)-C(21)	1.359(3)	N(3)-N(4)-C(22)	119.0(2)
N(4)-C(22)	1.453(3)	N(3)-C(15)-C(16)	112.6(2)
C(1)-C(2)	1.459(3)	N(4)-C(21)-C(16)	107.0(2)
C(15)-C(16)	1.414(3)	C(15)-C(16)-C(21)	103.3(2)
C(22)-C(23)	1.481(5)	C(15)-N(3)-N(4)	105.5(1)
		C(21)-N(4)-N(3)	111.5(1)
		C(21)-N(4)-C(22)	128.5(2)

The supplementary crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC deposition number 756955). Copies can be obtained, free of charge,

from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(1223) 336033; e-mail: (deposit@ccdc.cam.ac.uk).

Biology

The antimicrobial effects against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *Candida albicans* ATCC 10213 as well as the antiproliferative activity against NCI-H460 (human non-small-lung cancer), HT-29 ($COX2^{+/+}$, $p53^{-/-}$)(human colon adenocarcinoma), HCT116 ($COX2^{-/-}$, $p53^{+/+}$) (human colon cancer) and MCF-7 (breast cancer) cell lines were performed by general methods previously described.[22,23]

The ability of the test compound to inhibit the conversion of arachidonic acid to prostaglandin H_2 (PGH_2) was determined using a COX1/COX2 inhibitor screening assay kit (cat. n#560101;Cayman Chemical, Ann Arbor, MI).

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