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Novel Benzoylurea Derivatives as Potential Antitumor Agents; Synthesis, Activities and Structure-Activity Relationships

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A series of pyrazoloxyphenyl benzoyl urea derivatives was designed and synthesized for cytotoxic evaluation as potential antitumor agents. The synthetic compounds were evaluated for in vitro cytotoxicity against five human tumor cell lines, including A-549, SKOV-3, SK-MEL-2, XF-498 and HCT-15. Among others, compound **11** exhibited 50~100 times greater antitumor activities than the commercial product, Cisplatin.

Key words: Benzoyl Urea, Anticancer, Cisplatin, Cytotoxicity

INTRODUCTION

A variety of phenylbenzoylurea derivatives are well known as insecticides which inhibit the chitin synthesis of insects, and some of them are commercially available. (Wright *et al.*, 1987). Chlorfluazuron (**1**) is a representative commercial product which is widely used as insecticide. While chlorfluazuron itself had no antitumor activity, similar benzoylurea compound **2** was reported to possess antitumor activity against p388 leukemia (Okada *et al.* 1991). There are also several relevant reports (Howbert *et al.*, 1990, Chern *et al.*, 1997) about the diarylsulfonylurea compounds **3** which were reported to be effective against solid tumors.

After the report by Okada *et al.*, various benzoylurea compounds were further investigated in the search of potential anticancer derivatives to come up with HO-221 (**4**) by Ishihara Shngyo Kaisha, LTD. (Kondo *et al.*, 1993). Although HO-221 was found to act as a strong inhibitor of DNA polymerase alpha activity and selectied as a candidate compound to be developed, its inherent solubility property has caused it to be viewed as inappropriate for use in medicinal drug. The careful scrutinity of HO-221 by comparing with the earlier lead compound **2** revealed that the heterocyclclic groups attached to phenyl ring affect the anticancer activity dramatically.

In continuation of our work on benzoylurea compounds (Hwang *et al.*, 1995 and 1997) and in view of above mentioned literature precedence, it was planned to undertake the synthesis of benzoylurea compounds containing novel pyrazole heterocylic moiety to secure compounds of enhanced antitumor activities. The present paper describes synthesis and biological evaluation of the benzoylurea derivatives containing novel pyrazole moieties.

MATERIAL AND METHODS

Synthesis

Melting points were recorded on electrothermal melting point apparatus and are uncorrected. Mass spectra were recorded on a Shimadzu QP-1000 spectrometer (20 eV). ^{1}H-NMR and ^{13}C-NMR data were obtained from Jeol 400 MHz spectrometer and chemical shifts (δ) were reported in ppm in relation to tetramethylsilane (δ 0.00) and $CDCl_3$ (δ 77.0) for ^{1}H and ^{13}C NMR, respectively. Thin layer chromatography were performed on pre-coated silica gel 60 F-254 (layer thickness 0.2 mm, Merck). The Flash Column Chromatography was performed on Merck silica gel type 60 (230~400 mesh). The organic solvents and chemicals were obtained from Aldrich. Co. and purified by the appropriate methods before use.

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General procedure for the preparation of compound 7

To a stirred solution of commercially available chloronitrobenzene (**5**, 10mmol) and pyrazole (**6**, 10 mmol) in dimethylformamide (10 mL) was added potassium carbonate (15 mmol). The reaction mixture was stirred at 90°C~100°C for 3 h. After stirring the whole mixture at the reaction mixture at 90°C~100°C for 3 h, the reaction mixture was diluted with water (30 mL), and then extracted with ethyl acetate (15 mL × 3). The combined ethylacetate solution was washed with brine, dried over $MgSO_4$ anc concentrated in vacuo to provide crude products as solid. The crude products were washed with hexane to give essentially pure materials. Analytical sample can be prepared by flash column chromatography upon needed.

3,5-Dichloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl) oxy-nitrobenzene (7a). Yeld 70%; mp 138-140°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 5.6 (s,1H), 8.3 (s,2H); MS : m/z 354 (M^+).

3-Chloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl)oxy-nitrobenzene (7c). Yield 73%; mp 120-123°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 6.2 (s, 1H), 7.3 (d, 1H), 8.0-8.3 (m, 2H); MS : m/z 321 (M^+).

3-Trifluoromethyl-4-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)oxy-nitrobenzene (7e). Yield 65%; mp 134-136°C; ^{1}H NMR ($CDCl_3$) δ 6.4 (s, 1H), 7.1-7.8 (m, 6H), 8.5-8.7 (m, 2H); MS : m/z 417 (M^+).

2,6-Dichloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl) oxy-nitrobenzene (7f). Yeld 40%; mp 144-145°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 6.1 (s, 1H), 7.3 (s, 1H), 8.3 (s, 1H); MS: m/z 354 (M^+).

3-Chloro-4-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)oxy-nitrobenzene (7g). Yield 76%; mp 164-166°C; ^{1}H NMR ($CDCl_3$) δ 6.3 (s, 1H), 7.2-7.7 (m,6H), 8.4-8.9 (m,2H); MS : m/z 383 (M^+).

3,5-Dichloro-4-(1-methyl-3-trifluoromethyl-4-pyrazoyl) oxy-nitrobenzene (7h). Yeld 71%; mp 163-164°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 7.0 (s,1H), 8.2 (s,2H); MS : m/z 354 (M^+).

3-Chloro-(4-1-methyl-3-trifluoromethyl-4-pyrazoyl) oxy-nitrobenzene (7i). Yield 76%; mp 144-146°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s,3H), 7.0 (s,1H), 7.3 (d,1H), 8.0-8.3 (m,2H); MS : m/z 321 (M^+).

General Procedure for the Preparation of Compound 8

A flask containing compound **7** (3 mmol), methanol (30 mL) and Raney nickel (0.05 g) was equipped with a rubber balloon inflated with hydrogen gas. The reaction mixture was heated to 90°C for 4 h, cooled to rt, filtered to remove Raney nickel and than concentrated to give crude product as a solid, which was purified by flash column chromatography with EtOAc/Hexane (1/5) as eluent to provide pure product.

3,5-Dichloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl) oxy-aniline(8a). Yeld 92%; mp 125-126°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 3.9 (s, 3H), 5.5 (s, 1H), 6.7 (s, 2H); MS : m/z 324 (M^+).

3-Chloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl)oxy-aniline (8c). Yield 90%; mp 110-112°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 3.9 (s,3H), 5.7 (s,1H), 6.7-7.1 (m, 3H); MS : m/z 291 (M^+).

3-Trifluoromethyl-4-(1-phenyl-3-trifluoromethyl-5-pyrazoyl) oxy-aniline (8e). Yield 58%; mp 120-123°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 5.8 (s, 1H), 6.5-8.1(m, 8H); MS : m/z 387 (M^+).

2,6-Dichloro-4-(1-methyl-3-trifluoromethyl-5-pyrazoyl) oxy-aniline (8f). Yeld 92%; mp 130-131°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 3.9 (s, 3H), 5.7 (s, 1H), 6.4 (s, 1H); MS : m/z 324 (M^+).

3-Chloro-4-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)oxy-aniline (8g). Yield 88%; mp 150-151°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 5.7 (s, 1H), 6.5-7.8 (m,8 H); MS : m/z 353 (M^+).

3,5-Dichloro-4-(1-methyl-3-trifluoromethyl-4-pyrazoyl) oxy-aniline (8h). Yeld 94%; mp 136-138°C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3H), 5.2 (s, 2H), 6.9 (s, 2H), 7.2 (s, 1H); MS : m/z 324 (M^+).

3-Chloro-4-(1-methyl-3-trifluoromethyl-4-pyrazoyl)oxy-aniline (8i). Yield 91%; mp 128-130°C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 2H), 3.9 (s, 3H), 6.4-7.0 (m, 4H); MS : m/z 291 (M^+).

General Procedure for the Preparation of Compound 11

A solution of 2-nitrobenzamide (2 mmol) and oxazolyl chloride (2 mmol) in 1,2-dichloroethane (8 mL) was heated at 100°C for 2 h under argon atmosphere. To the resulting isocyanate solution was added compounds **8** at rt. After stirring for 1 h, the reaction mixture was concentrated under vacuo and the residue was chromatographed with EtOAc/Hexane (1/5) as eluent to provide pure product

Table I Chemical Structures and Physical Properties of Compound **8**

No.	W	X	Formula	Y	mp,	yield,%	^{1}H NMR(acetone-d_6,)
11a	4H	CH_3	$C_{19}H_{12}Cl_2F_3N_3O_5$	3,5-Cl_2	225-226	70	3.9(s,3H), 5.8(s,1H), 7.8-8.3(m,6H), 10.5(s,1H), 10.7(s,1H)
11b	4H	t-butyl	$C_{22}H_{18}Cl_2F_3N_5O_5$	3,5-Cl_2	228-229	65	1.8(s, 9H), 5.8(s,1H), 7.8-8.3(m,7H), 10.5(s,1H), 10.7(s, 1H)
11c	4H	CH_3	$C_{19}H_{13}ClF_3N_5O_5$	3-Cl	205-206	64	3.9(s,3H), 5.9(s,1H), 7.4-8.3(m,7H), 10.4(s,1H), 10.6(s,1H)
11d	4H	t-butyl	$C_{22}H_{19}ClF_3N_5O_5$	3-Cl	195-197	66	1.8(s, 9H), 5.9(s,1H), 7.4-8.3(m,7H), 10.4(s,1H), 10.6(s,1H)
11e	4H	Phenyl	$C_{25}H_{15}F_6N_5O_5$	3-CF_3	153-154	62	6.3(s,1H), 7.4-8.3(m,12H), 10.4(s,1H), 10.7(s,1H)
11f	4H	CH_3	$C_{19}H_{12}Cl_2F_3N_5O_5$	2,5-Cl_2	208-209	70	3.9(s,3H), 6.1(s,1H), 7.7-8.7(m,6H), 10.6(s,1H), 11.2(s,1H)
11g	4H	Phenyl	$C_{24}H_{15}ClF_3N_5O_5$	3-Cl	212-214	74	6.1(s,1H), 7.5-8.3(m,12H), 10.4(s,1H), 10.6(s,1H)
11h	5H	CH_3	$C_{19}H_{12}Cl_2F_3N_5O_5$	3,5-Cl_2	253-254	73	3.8(s,3H), 7.3(s,1H), 7.8-8.3(m,7H), 10.4(s,1H), 10.6(s,1H)
11i	5H	CH_3	$C_{19}H_{13}ClF_3N_5O_5$	3-Cl	201-202	71	3.9(s,3H),7.1-8.3(m,8H), 10.4(s,1H), 10.5(s,1H)

in 62-75% yields.

2-n tro-N-[[[3,5-dichloro-4-(1-metyl-3-trifloromethyl-5-pyrazoyl)oxyphenyl]amino]carbonyl] benzdmide (11a). Yield 70%; mp 225; ^{1}H NMR (*aceton-d*$_6$) δ 3.9 (s, 3H), 5.8 (s, 1H), 7.8-8.3 (m, 6H), 10.5 (s, 1H), 10.7 (s, 1h); MS : m/z 518 (M+).

The chemical structures and their physical properties for other compounds **11** are summarized in Table I.

Anticancer activity test (*in vitro*)

Anticancer assay was performed by Pharmaceutical Screening Laboratory in Korea Research Institute of Chemical Technology using five different human tumor cell lines, A-549 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), HCT-15 (human colon), XF-498 (human CNS) which were purchased from the National Cancer Institute (NCI) in U.S.A.

The cells were grown at 37°C in RPMI 1640 medium supplemented with 10% FBS and separated using PBS containing 0.25% trypsin and 3 mM EDTA. 5×10^3-2×10^4 cells were added to each well of 96 well plate and incubated at 37°C for 24 h. Each compound was dissolved in DMSO and diluted with the above medium at five different concentrations with the range of 0.1-30 μg/mL. The DMSO concentration was set to be below 0.5% and filtrated using 0.22 mg filter. After removing the well medium by aspiration, a 200 μL portion of the solution was added to above well plates which were placed in 5% CO_2 incubator for 48 h. The protein stain assay was performed according to SRB method (Skehan *et al.*, 1990).

RESULTS AND DISCUSSION

Chemistry

The synthesis of target compounds **11** was started from the preparation of pyrazoyloxynitrobenzene **7** by condensing commercially available chloronitrobenzene **5** with pyrazoles **6** under basic condition at 90°C~100°C for 3 h in 65~75% yield (Scheme 1). The required 5-hydroxy-3-trifluoromethylpyrazole and 4-hydroxy-3-trifluoromethylpyrazole was prepared utilizing the previously described methods by Bruce *et al.*, 1993 and Iwata *et al.*, 1991, respectively.

The nitrocompounds **7** were then reduced to aniline analogues by treating them under normal hydrogenation condition. With the key intermediates **8** in hand, the final

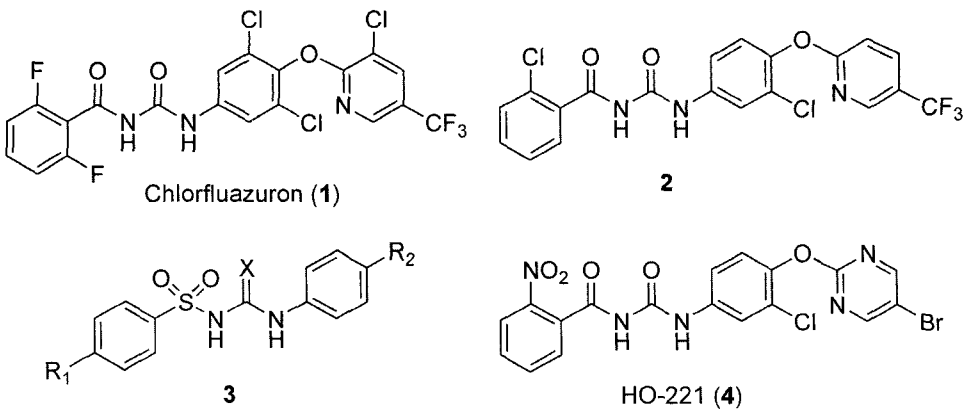
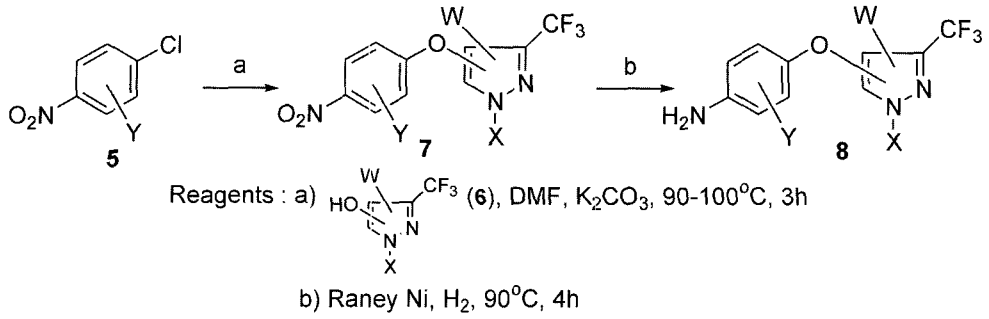
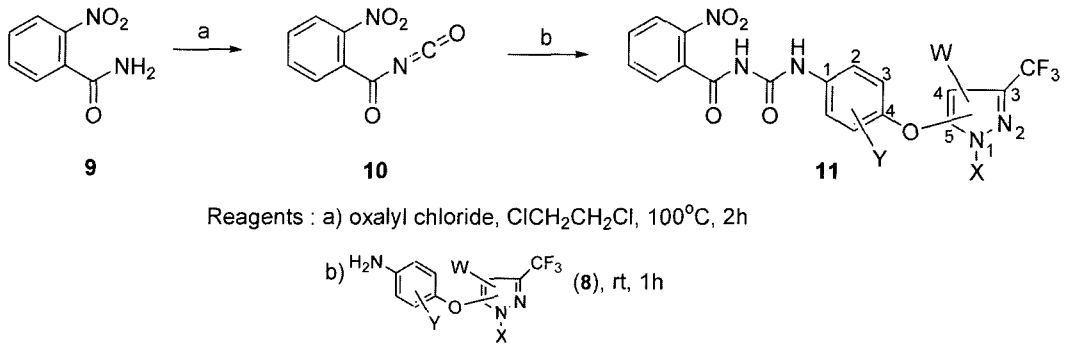


Fig. 1. Structures of some biologically active urea compounds



Scheme 1. Synthesis of Key Intermediates Pyrazoyloxyanilines **8**



Scheme 2. Synthesis of Benzoyl Urea Derivatives **11**

products **11** were prepared with no incidence by reacting **8** with nitrobenzoyl isocyanate **9** which was formed in situ from nitrobenzamide and oxalyl chloride (Scheme 2).

Anticancer assay

The antitumor activities of the compound **11** toward five different human tumor cells was determined from the Tz (number of cells before the addition of the test compounds), C (number of cells after 48 h incubation without test compounds), and T (number of cells after 48h incubation with test compounds) by the following equation (1) or (2).

$$\text{If } Tz>T, \quad \left[\frac{(T-Tz)}{(C-Tz)}\right]\times 100 \qquad (1)$$

$$\text{If } Tz<T, \quad \left[\frac{(T-Tz)}{Tz}\right]\times 100 \qquad (2)$$

The value thus obtained were applied to Lotus program using data regression to five ED_{50} of each compound. The result of the in vitro anticancer data are compared to commercial product cisplatin (Sigma Tau Co, Italy) and summarized in Table II.

The results indicate excellent antitumer activities toward the five different cell lines, A-549 (lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (malignant melanoma), XF-498 (CNS tumor) and HCT-15 (colon adenocarcinoma). Additionally compound 11 g exhibits 50~100 times more potent than commercial product cisplatin

Table II. Antitumuor Activites of target compound **11**

Compound No.[a]	ED_{50} (μg/mL)				
	A-549	SK-OV-3	SK-MEL-2	XF 498	HCT15
Cisplatin[b]	0.775	0.460	0.460	0.638	1.100
11a	0.281	0.332	0.242	0.494	0.202
11b	4.795	24.314	7.245	7.225	7.489
11c	0.256	0.281	0.139	0.129	0.022
11d	2.611	4.284	2.386	4.431	2.701
11e	1.137	1.597	0.640	1.763	0.880
11f	0.614	1.212	0.429	2.059	0.383
11g	0.916	3.301	1.059	2.282	0.951
11h	2.993	3.041	1.960	3.644	1.929
11i	0.011	0.023	0.022	0.029	0.013

[a] refer to Table I.
[b] Italy, Sigma Tau

toward the five human cancer cell lines. Now it can be clearly stated that benzoylurea derivatives containing heterocyclic pyrazol group possess excellent antitumor activities in vitro, and further study including in vivo experiments and molecular modifications is warranted.

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