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Synthesis of spiro[indolo-1,5-benzodiazepines] from 3-acetyl coumarins for use as possible antianxiety agents

RAVIRAJ A KUSANUR[1], MANJUNATH GHATE[2] and MANOHAR V KULKARNI[1]*
[1]Department of Studies in Chemistry, Karnataka University, Dharwad 580 003, India
[2]Kripanidhi College of Pharmacy, Bangalore, India
e-mail: drmvk274@yahoo.co.in

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Abstract. 3-Acetyl coumarins (**1**) when allowed react with isatin (**2**) gave corresponding 3-(3′-hydroxy-2′-oxo indolo) acetyl coumarins (**3**), which on dehydration afforded the corresponding *a*,*b*-unsaturated ketones (**4**). Cyclocondensation of (**4**) with substituted *o*-phenylene diamines resulted in novel 3-coumarinyl spiro[indolo-1,5-benzodiazepines] (**5**). Structures of all the compounds have been established on the basis of their IR, NMR and mass spectral data and have been screened for their antimicrobial activity and antianxiety activity in mice.

Keywords. Spirobenzodiazepines; distereotopic protons; antianxiety activity.

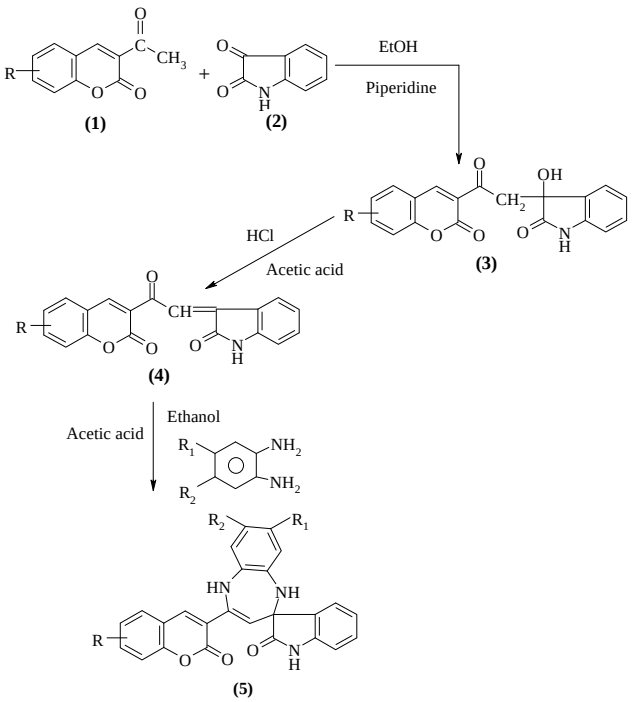
1. Introduction

1,5-Benzodiazepines constitute an important class of psychopharmaca,[1] in particular as tranquilizers and also as potent virucides and non-nucleoside inhibitors of HIV-1 reverse transcriptase.[2] Beside this, 1,5-benzodiazepines show antifungal, antibacterial,[3] antifeedant,[4] anti-inflammatory, analgesic[5] and anticonvulsant[6] activities. The fusion of a heterocyclic system to the benzodiazepine ring appears quite promising for the synthesis of derivatives with greater activity and specificity. Coumarins containing nitrogen heterocycles at C_3 position are used as dyes.[7] They are also used in the manufacture of printed circuits.[8] Here we report the synthesis of spiro indolo benzodiazepines from 3-acetyl coumarins. The results of the evaluation of antimicrobial and antianxiety activities of these compounds are also reported.

2. Results and discussion

3-Acetyl coumarins[9,10] (**1**) selectively reacted with the C_3-carbonyl of isatin in presence of piperidine to give the *b*-hydroxycarbonyl compounds (**2**). These underwent dehydration under acidic conditions to generate the orange coloured *a*,*b*-unsaturated carbonyl compounds (**4**). Cyclo condensation of compounds (**4**) with o-phenylenediamines resulted in the formation of spiro benzodiazepines (**5**) (scheme 1).

The IR spectrum of compound **3a** (R = H) showed three strong bands at 1735, 1707 and 1688 cm^{-1} due



R = H, 6-CH_3, 8-OCH_3, 5,6-benzo, 6-Cl, 6-Br; R_1=R_2=H, CH_3

Scheme 1.

*For correspondence

Table 1. IR and PMR spectral data of compounds (**3a–f**).

Compd.	R	IR (cm^{-1})					PMR *d* (ppm)
		$n_{C=O\ (lactone)}$	$n_{C=O\ (keto)}$	$n_{C=O\ (amide)}$	n_{NH}	n_{OH}	
3a	H	1735	1707	1688	3207	3491	3·58 (*d*, 2H, *J* = 17·49 Hz), 4·13 (*d*, 2H, *J* = 17·4 Hz), 7·63 (*b*, *s*, 1H, NH, D_2O exchangable), 3·71 (*s*, 1H, OH, D_2O exchangable), 8·53 (*s*, 1H, C_4-H), 6·89–7·7 (*m*, 8H, Ar-H)
3b	6-CH_3	1722	1701	1683	3190	3404	2·41 (*s*, 3H, C_6-CH_3), 3·59 (*d*, 2H, *J* = 17·5 Hz), 4·13 (*d*, 2H, *J* = 17·6 Hz), 7·7 (*b*, *s*, 1H, NH, D_2O exchangable), 3·73 (*s*, 1H, –OH, D_2O exchangable), 8·45 (*s*, 1H, C_4-H), 6·87–7·47 (*m*, 7H, Ar-H)
3c	8-OCH_3	1727	1705	1680	3352	3409	3·96 (*s*, 3H, 8-OCH_3), 3·68 (*d*, 2H, *J* = 17·7 Hz), 4·07 (*d*, 2H, *J* = 17·8 Hz), 10·2 (*s*, 1H, NH, D_2O exchangable), 3·71 (*s*, 1H, OH, D_2O exchangable), 8·37 (*s*, 1H, C_4-H), 6·81–7·32 (*m*, 7H, Ar-H)
3d	5,6-Benzo	1727	1701	1672	3218	3382	4·22 (*d*, 2H, *J* = 17·91 Hz), 3·81 (*d*, 2H, *J* = 17·8 Hz), 9·91 (*s*, 1H, NH, D_2O exchangable), 3·67 (*s*, 1H, OH, D_2O exchangable), 9·13 (*s*, 1H, C_4-H), 6·88–8·29 (*m*, 10H, Ar-H)
3e	6-Cl	1725	1725	1681	3192	3371	4·31 (*d*, 2H, *J* = 17·61 Hz), 3·72 (*d*, 2H, *J* = 17·5 Hz), 9·12 (*s*, 1H, NH, D_2O exchangable), 3·81 (*s*, 1H, OH, D_2O exchangable), 9·12 (*s*, 1H, C_4-H), 6·81–7·46 (*m*, 7H, Ar-H)
3f	6-Br	1729	1730	1686	3204	3381	4·34 (*d*, 2H, *J* = 17·84 Hz), 3·81 (*d*, 2H, *J* = 17·7 Hz), 8·84 (*s*, 1H, NH, D_2O exchangable), 3·74 (*s*, 1H, OH, D_2O exchangable), 9·14 (*s*, 1H, C_4-H), 6·79–7·48 (*m*, 7H, Ar-H)

to lactone, ketone and amide carbonyls respectively. In the PMR spectrum of **3a** (R = H), the diastereotopic CH_2 protons appeared as two separate doublets at 3·50 and 4·13 ppm (*J* = 17·5 Hz). The NH and OH protons were characterized by D_2O exchange. The other protons resonated at expected fields (table 1).

The *b*-hydroxy ketones (**3**) readily underwent dehydration in presence of an acid to yield *a*,*b*-unsaturated ketones (**4**). The formation of enones was characterized by the absence of OH stretching and the shift of keto carbonyl stretching to lower frequency at ~1620 cm^{-1} in their IR spectra. The IR spectrum of compound **4a** (R = H) shows band at 1721 cm^{-1} due to $n_{C=O}$ of lactone and bands at 1610 and 1622 cm^{-1} due to $n_{C=O}$ of keto and $n_{C=O}$ of amide respectively. The PMR spectrum of compound **4a** (R = H) showed a singlet at 7·93 ppm due to olefinic proton and singlet at 8·70 ppm due to C_4-H of coumarin. Aromatic protons resonated in the region 6·96–7·83 ppm. The NH proton was observed at 10.46 ppm, which was confirmed by D_2O exchange (table 2).

a,*b*-Unsaturated ketones (**4**) when treated with *o*-phenylene diamine in ethanol afforded spirobenzodiazepines (**5**). In the IR spectrum of compound **5a** (R = H, R_1 = R_2 = H) the N–H stretching band observed at 3435 cm^{-1} (table 3). The absence of keto-carbonyl stretching confirms the conversion of *a*,*b*-unsaturated ketones into spirobenzodiazepines (**5**). Lactone and amide carbonyls appeared at 1716 and 1673 cm^{-1} respectively. The PMR spectrum of com-

Table 2. IR and PMR spectral data of compounds **4**(**a–f**).

Compd.	R	IR (cm^{-1})				PMR *d* (ppm)
		$n_{C=O\ (lactone)}$	$n_{C=O\ (keto)}$	$n_{C=O\ (amide)}$	n_{N-H}	
4a	H	1721	1610	1662	3188	7·93 (*s*, 1H, =CH), 8·70 (*s*, 1H, C_4-H), 10·46 (*s*, 1H, NH, D_2O exchangable), 7·83 (*d*, 1H, C_5-H, *J* = 7·48 Hz), 7·73 (*t*, 1H, C_6-H, *J* = 8·3 Hz), 7·40 (*t*, C_7-H, *J* = 8·1 Hz), 8·45 (*d*, 1H, C_8-H, 7·74 Hz), 6·87 (*d*, 1H, $C_{4'}$-H, *J* = 7·8 Hz), 6·96 (*t*, 1H, C_5'-H, *J* = 7·34 Hz), 7·40 (*t*, 1H, C_6'-H, *J* = 7·21), 7·42 (*d*, 1H, C_7'-H, *J* = 7·2 Hz)
4b	6-CH_3	1727	1622	1661	3196	2·46 (*s*, 3H, C_6-CH_3), 7·94 (*s*, 1H, =CH), 8·59 (*s*, 1H, C_4-H), 10·33 (*s*, 1H, NH, D_2O exchangable), 7·57 (*s*, 1H, C_5-H), 7·52 (*d*, 1H, C_7-H, *J* = 7·8 Hz), 8·46 (*d*, 1H, C_8-H, *J* = 7·66 Hz), 6·86 (*d*, 1H, C_4'-H, *J* = 7·6 Hz), 6·37 (*t*, 1, C_5'-H, *J* = 7·6 Hz), 7·31 (*t*, 1H, C_6'-H, *J* = 7·7 Hz), 7·53 (*d*, 1H, C_7'-H, *J* = 7·6 Hz)
4c	8-OCH_3	1733	1606	1655	3306	4·00 (*s*, 3H, 8-OCH_3), 7·93 (*s*, 1H, =CH), 8·64 (s, 1H, C_4-H), 10·40 (1H, NH, D_2O exchangable), 6·85–8·47 (*m*, 7H, Ar-H)
4d	5,6-Benzo	1727	1601	1645	3186	10·56 (*s*, 1H, D_2O exchangable), 9·46 (*s*, 1H, C_4-H), 7·90 (*s*, 1H, =CH), 6·87–8·47 (*m*, 7H, Ar-H)
4e	6-Cl	1724	1628	1676	3371	7·96 (*s*, 1H, =CH), 8·71 (*s*, 1H, C_4-H), 10·50 (1H, NH, D_2O exchangable), 6·47–7·96 (*m*, 7H, Ar-H)
4f	6-Br	1729	1641	1669	3324	7·88 (*s*, 1H, =CH), 7·69 (*s*, 1H, C_4-H), 10·46 (1H, NH, D_2O exchangable), 6·5–8·10 (*m*, 7H, Ar-H)

pound **5a** (R = H, $R_1 = R_2$ = H) shows a singlet at 8·87 ppm due to C_4-H of coumarin (table 3). The olefinic proton of benzodiaepine resonated as singlet at 8·93 ppm, whereas aromatic protons resonated as multiplet at 7·28–8·16 ppm.

EI mass spectrum of compound **5a** (R = H, $R_1 = R_2$ = H) *m*/*z* 407 (M^+ 5%), 274 (100%), 246 (35%), 219 (11%), 190 (10%), 172 (8%), 69 (30%) confirmed the formation of benzodiazepins.

3. Biological activity

3.1 *Antimicrobial activity*

Antimicrobial activity was carried out against two pathogenic bacteria *E. coli*, and *B. subtillis*, and *A. niger* as the fungal strain. The reference drugs used were *ciprofloxacin* and *griseofulvin* respectively. The tests were carried out by cup plate method.[11] Among all the benzodiazepino coumarins **5**, **5f** with R = 8-OCH_3. $R_1 = R_2 = CH_3$ show 88·88% inhibition against *B. substillis* and 77·77% of inhibition against *E. coli* as compared to the standard and other compounds are moderately active. Compounds **5b** and **5i** show 77·77% of inhibition against *A. niger* and other compounds are moderately active. The results are shown in table 4.

3.2 *Antianxiety activity in mice*

All the newly synthesised benzodiazepines were screened for their antianxiety activity in mice on plus maze apparatus devised by Crawley and Godwin,[12] modified by T Kilofoil[13] using sodiumpentabarbitone as the standard.

The apparatus consists of plexiglass box ($40 \times 21 \times 21$ inches) divided into two chambers by black plexiglass partition. The box was placed within a layer of soundproof box, which is equipped with one-way observation window. The partition dividing the two chambers 13×5 inch opening, through which animal could easily pass. The dark chamber

Table 3. IR and PMR spectral data of compounds **5(a–l)**.

Compd.	R	R_1	R_2	IR (cm^{-1}) $n_{C=O\ (lactone)}$	IR (cm^{-1}) $n_{C=O\ (amide)}$	IR (cm^{-1}) n_{N-H}	PMR *d* (ppm)
5a	H	H	H	1716	1673	3435	8·87 (*s*, 1H, C_4-H), 9·83 (*s*, 1H, =CH), 7·28–8·16 (*m*, 12H, Ar-H)
5b	H	CH_3	CH_3	1701	1632	3272	2·52 (*s*, 6H, $-CH_3$), 8·80 (*s*, 1H, C_4-H), 9·71 (*s*, 1H, =CH), 7·26–7·89 (*m*, 10H, Ar-H)
5c	6-CH_3	H	H	1705	1629	3399	2·45 (*s*, 3H, C_6-CH_3), 8·78 (*s*, 1H, C_4-H), 9·80 (*s*, 1H, =CH), 7·26–7·90 (*m*, 11H, Ar-H)
5d	6-CH_3	CH_3	CH_3	1716	1671	3404	2·45 (*s*, 3H, C_6-CH_3), 2·52 (*s*, 6H, $-CH_3$), 8·75 (*s*, 1H, C_4-H), 9·70 (*s*, 1H, =CH), 7·26–7·91 (*m*, 9H, Ar-H)
5e	8-OCH_3	H	H	1712	1656	3427	4·02 (*s*, 3H, 6-OCH_3), 8·84 (*s*, 1H, C_4-H), 9·84 (*s*, 1H, =CH), 7·15–8·19 (*m*, 11H, Ar-H)
5f	8-OCH_3	CH_3	CH_3	1722	1694	3399	2·53 (*s*, 6H, CH, CH_3), 4·02 (*s*, 3H, C_6-OCH_3), 8·80 (*s*, 1H,C_4-H), 9·74 (*s*, 1H, =CH), 7·14–7·94 (*m*, 9H, Ar-H)
5g	5,6-Benzo	H	H	1716	1651	3420	9·68 (*s*, 14H, C_4-H), 9·84 (*s*, 1H, =CH), 6·92–8·58 (*m*, 14H, Ar-H)
5h	5,6-Benzo	CH_3	CH_3	1716	1662	3404	2·56 (*s*, 6H, CH_3), 9·32 (*s*, 1H, C_4-H), 9·80 (*s*, 1H, =CH), 6·92–8·58 (*m*, 12H, Ar-H)
5i	6-Cl	H	H	1712	1684	3394	8·91 (*s*, 1H C_4-H), 9·83 (*s*, 1H, =CH), 7·29–8·2 (*m*, 11H, Ar-H)
5j	6-Cl	CH_3	CH_3	1713	1640	3328	2·54 (*s*, 6H, CH_3), 8·92 (*s*, 1H, C_4-H), 9·86 (*s*, 1H, =CH), 7·34–8·4 (*m*, 9H, Ar-H)
5k	6-Br	H	H	1704	1639	3298	8·94 (*s*, 1H, C_4-H), 9·84 (*s*, 1H, =CH), 7·29–8·40 (*m*, 11H, Ar-H)
5l	6-Br	CH_3	CH_3	1708	1646	3204	2·56 (*s*, 6H, CH_3), 8·86 (*s*, 1H, C_6-H), 9·86 (*s*, 1H, =CH), 7·34–8·4 (*m*, 9H, Ar-H)

(14 × 21 × 21 inch) was made up of dark plexiglass except for the side ferry observation window. This side is clear and covered with black plastic. The testing was performed between 12·00 noon to 6·00 p.m. in an isolated darkened laboratory.

Mice weighing 25 g were chosen and were sorted into five animals in a group. They were allowed free access to food, water and libitum. Animals were given 60 min time to acclimatize to the environment prior to the administration of drugs. Drugs or test samples in DMF were given at the dose of 20 mg/kg body weight from here each animal was individually placed in the centre of the light area of the apparatus and observed for 10 min. The total amount of time spent in dark area is measured. Lesser the time spent in dark space the greater is the antianxiety activity of the drugs. The results are given in table 5. Compound **5a** with R = H, R_1 = R_2 = H have shown comparable activity with the standard and the other compounds are moderately active.

4. Experimental

Melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked by TLC. IR spectra were recorded in KBr on Perkin–Elmer spectrophotometer (*n* in cm^{-1}) and PMR spectra on Bruker-300 MHz FTNMR spectrometer using TMS as internal standard (chemical shifts in *d*, ppm). Mass spectra were recorded on a Jeol JMS D-300 instrument at 70 ev.

4.1 *3-(3ℓ-hydroxy-2ℓ-oxo indolo) acetyl coumarins* **3(a–e)**

A mixture of 3-acetyl coumarin (1·88 g, 0·01 mole), isatin (1·47 g, 0·01 mole) in absolute alcohol (100 ml) and two drops of piperidine was stirred for half an hour at room temperature. The reaction mixture was allowed to stand for overnight at room temperature. The solid separated was filtered and

Table 4. Antimicrobial activity of compounds **5(a–l)**.

				B. subtillis		*E. coli*		*A. niger*	
Compd.	R	R_1	R_2	Zone of inhibition (mm)	Relative inhibition (%)	Zone of inhibition (mm)	Relative inhibition (%)	Zone of inhibition (mm)	Relative inhibition (%)
5a	H	H	H	18	66·66	17	61·11	15	50·00
5b	H	CH_3	CH_3	16	55·55	17	61·11	20	77·77
5c	6-CH_3	H	H	12	33·33	10	22·22	12	33·33
5d	6-CH_3	CH_3	CH_3	10	22·22	10	22·22	10	22·22
5e	8-OCH_3	H	H	18	66·66	17	61·11	18	66·66
5f	8-OCH_3	CH_3	CH_3	22	88·88	20	77·77	20	77·77
5g	5,6-Benzo	H	H	18	61·11	16	55·55	15	50·00
5h	5,6-Benzo	CH_3	CH_3	18	61·11	10	22·22	12	33·33
5i	6-Cl	H	H	18	66·66	19	72·22	20	77·77
5j	6-Cl	CH_3	CH_3	17	61·11	16	55·55	14	44·44
5k	6-Br	H	H	18	66·66	19	72·22	16	55·55
5l	6-Br	CH_3	CH_3	17	61·11	16	55·55	16	55·55
DMF				06	–	06	–	06	–
Ciprofloxacin				24	100	24	100	–	–
Griseofulvin				–	–	–	–	24	100

Table 5. Results of antianxiety activity in mice.

Compd.	R	R_1	R_2	Time spent in dark space (s)
5a	H	H	H	308 ± 12·0
5b	H	CH_3	CH_3	320 ± 16·8
5c	6-CH_3	H	H	318 ± 18·5
5d	6-CH_3	CH_3	CH_3	358 ± 15·0
5e	8-OCH_3	H	H	314 ± 12·0
5f	8-OCH_3	CH_3	CH_3	315 ± 09·8
5g	5,6-Benzo	H	H	316 ± 08·9
5h	5,6-Benzo	CH_3	CH_3	319 ± 11·1
5i	6-Cl	H	H	324 ± 12·1
5j	6-Cl	CH_3	CH_3	328 ± 12·1
5k	6-Br	H	H	318 ± 09·4
5l	6-Br	CH_3	CH_3	321 ± 08·4
Control				401·3 ± 25·0
Pentabarbitone				295 ± 11·51

recrystallised from alcohol-dioxane mixture to afford the hydroxy compound **3a** (3·12 g, 93·13%).

4.2 *3-[2′-oxo-3′-indolo]a,b-unsaturated ketones* **4(a–e)**

To the solution of 3-(3′-hydroxy-2′-oxo indolo) acetyl coumarin (**3a**) (3·35 g, 0·01 mole) in acetic acid (25 ml), 0·5 ml of concentrated hydrochloric acid was added. The reaction mixture was warmed on water bath for one hour and cooled to room temperature. The separated orange coloured precipitate was filtered and recrystallised from alcohol dioxane mixture to afford the ketone **4a** (2·90 g, 91·48%).

4.3 *4′-(coumarin-3-yl)spiro[3H-indole-3,2′-1,5-benzodiazepine]-2(1H)-one*

To a solution of 3-[(2′-oxo-3′-indolo] *a,b*-unsaturated ketones (**4a**) (1·58 g 0·005 mole) in ethanol

Table 6. Physical and analytical data of compounds **5(a–l)**.

Compd.	R	R_1	R_2	Solvent	MP (°C)	Yield (%)	Mol. formula	Analysis found (calc.) (%) C	H	N
5a	H	H	H	Ethanol	183	70·88	$C_{25}H_{17}N_3O_3$	73·68 (73·71)	4·15 (4·17)	10·2 (10·31)
5b	H	CH_3	CH_3	Ethanol	178	69·11	$C_{27}H_{21}N_3O_3$	74·46 (74·48)	4·79 (4·82)	9·63 (9·65)
5c	6-CH_3	H	H	Ethanol	171	74·12	$C_{26}H_{19}N_3O_3$	74·05 (74·10)	4·48 (4·51)	9·94 (9·97)
5d	6-CH_3	CH_3	CH_3	Ethanol	215	72·30	$C_{28}H_{23}N_3O_4$	74·81 (74·85)	5·09 (5·12)	9·31 (9·35)
5e	8-OCH_3	H	H	Ethanol	208	65·81	$C_{26}H_{19}N_3O_4$	71·36 (71·39)	4·31 (4·34)	9·58 (9·61)
5f	8-OCH_3	CH_3	CH_3	Ethanol + dioxan	260	68·21	$C_{28}H_{23}N_3O_4$	72·21 (72·25)	4·71 (4·94)	9·00 (9·03)
5g	5,6-Benzo	H	H	Ethanol + dioxan	265	76·36	$C_{29}H_{23}N_3O_3$	75·43 (75·48)	4·95 (4·98)	9·08 (9·11)
5h	5,6-Benzo	CH_3	CH_3	Ethanol + dioxan	280	71·61	$C_{31}H_{27}N_3O_3$	76·01 (76·07)	5·49 (5·52)	8·49 (8·58)
5i	6-Cl	H	H	Ethanol	181	64·00	$C_{25}H_{16}N_3O_3Cl$	67·96 (68·02)	3·58 (3·62)	9·48 (9·52)
5j	6-Cl	CH_3	CH_3	Ethanol	192	62·20	$C_{27}H_{20}N_3O_3Cl$	69·03 (69·08)	4·21 (4·26)	8·91 (8·95)
5k	6-Br	H	H	Ethanol	198	63·52	$C_{25}H_{16}N_3O_3Br$	61·68 (61·72)	3·26 (3·29)	8·60 (8·64)
5l	6-Br	CH_3	CH_3	Ethanol	206	62·82	$C_{27}H_{20}N_3O_3Br$	63·48 (63·52)	3·09 (3·13)	8·19 (8·23)

(20 ml) was added *o*-phenylene diamine (0·60 g, 0·0055 mole) and 0·5 ml of acetic acid. The reaction mixture was refluxed for 10 h and cooled. The separated solid was filtered and recrystallised from alcohol dioxane mixture to afford the benzodiazepene (**5a**) (1·40 g, 70·88%) (table 6).

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