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# Structure function relationship study of yuehchukene. I. Anti-implantation and estrogenic activities of substituted yuehchukene derivatives

WL Chan[1], DD Ho[1], CP Lau[1], KH Wat[1], YC Kong[1]*, KF Cheng[2], TT Wong[2], TY Chan[2]

[1]*Biochemistry Department, The Chinese University of Hong Kong, Shatin, NT;*
[2]*Chemistry Department, The University of Hong Kong, Pokfulam Road, Hong Kong*

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**Summary** — (±)-Yuehchukene (YCK) is a novel bis-indole alkaloid with potent anti-implantation activity in rats. The present paper reports the activity of YCK derivatives by substitution on the parent compound. Substitutions on the indole nitrogens or on 2- and 5'-positions of the indole moiety abolished activity. *N*-1'-methylation of the free indole only permitted a 30% residual activity. Saturation of the $C_9$-$C_{10}$ cyclohexenyl double bond did not affect activity at all. Hydroxylation, whether on the $C_9$-$C_{10}$ double bond, or at $C_2$ or $C_5$, rendered the hydroxylated derivatives inactive. This suggests that the active metabolite in anti-implantation was probably not a hydroxylated derivative carrying estrogenic activity.

**Résumé — Relations structure–activité dans la série du yuehchukene. I. Les activités anti-nidation et œstrogénique des dérivés substitués du (±)-yuehchukene.** *Le (±)-yuehchukene (YCK) est un nouvel alcaloïde de type bis-indole avec une activité prononcée de type anti-nidation chez le rat. Cet article présente l'activité des dérivés de YCK obtenus à partir du composé original au moyen de diverses substitutions. La substitution des atomes d'azote indoliques, ou des carbones C-2, ou C-5' de la partie indolique supprime l'activité. La méthylation de l'atome d'azote de l'indole monosubstitué permet de conserver une activité résiduelle d'environ 30%. L'hydrogénation de la double liaison $C_9$-$C_{10}$ de la partie cyclohexénylique n'apporte aucun changement d'activité. Mais l'hydroxylation, soit de la double liaison $C_9$-$C_{10}$, soit des carbones C-2 ou C-5', conduit à des dérivés inactifs. Cela suggère que le métabolite responsable de l'activité de type anti-nidation n'est probablement pas un dérivé hydroxylé de structure apparentée à celle des œstrogènes.*

**yuehchukene (YCK) / non-steroidal anti-implantation agent / estrogenic activity**

(±)-Yuehchukene (YCK) is a novel bis-indole alkaloid; it has a unique linear tetracyclic indeno[2,1-*b*]indole basic unit with a free indole attached at the 6-position [1]. It was first isolated from the root of *Murraya paniculata* (L) Jack [2] but is now obtainable by synthesis [3]. YCK has potent anti-implantation activity in rats. However, YCK carried 8.2% and 4.5% estrogenicity in anti-implantation assay and uterotrophic assay respectively. This constitutes the main obstacle to consider developing YCK as a potential fertility regulating agent for human use.

Since YCK carries no oxygen function, its estrogenicity probably comes from a hydroxylated derivative through hepatic metabolism. While there is still no data indicating that the anti-implantation activity of YCK is a direct and quantitative manifestation of its estrogenecity, it may be worth trying to dissociate these two activities by making analogues which are more active in anti-implantation or analogues with diminished estrogenicity. In either case, it would make the inherent estrogenicity more tolerable at the effective anti-implantation dose level.

This paper concerns YCK analogues that were made in a first attempt to define the structural requirements for anti-implantation. A direct approach to obtain YCK analogues is by modification of the parent compound to yield substituted derivatives, or to start with modified isoprenyl indole precursors in Diels-Alder condensation [3].

Alternatively, synthesis of the tetracyclic unit permits the introduction of a free indole or a close congenor [4, 5]. Other synthetic approaches in progress are drastically departing from the three routes mentioned above; they yielded analogues with bond variations (in preparation), steric variation of the indane plant (Prof J Kutney, personal communications) and replacement of the indane moiety by other ring systems as well as synthesis of the enantiomers (in preparation).

*Correspondence and reprints

## Chemistry

The basic structural unit of yuehchukene (**1**) is the tetracyclic 7, 7, 9-trimethyl-5, 6, 6*a*, 7, 8, 10*a*-hexahydroindeno[2,1-*b*]indole (**13**) which is a novel structural feature in naturally occurring indole compounds. YCK differs from other bis-indole alkaloids in that it lacks a $(CH_2)_2N$ moiety at the 3-position of indole. In the present study of structure–activity relationship, all YCK derivatives retained the basic structure of the parent compound but with functional groups substituted at different positions. They were obtained by modification of the parent compound and dimerization of the modified precursors of YCK, leading to derivatives with the following structural features:

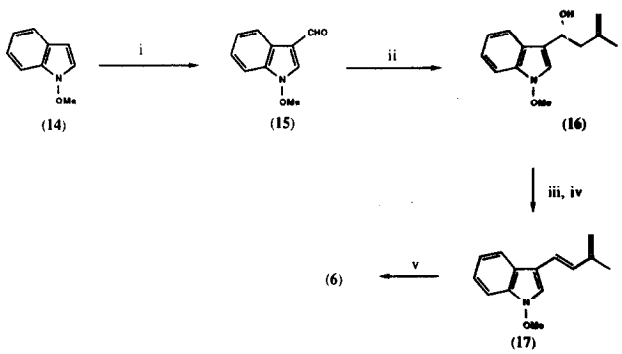
1) substitution at N-1' and N-5; 2) substitution at $C_2$, $C_5$, $C_9$ and $C_{10}$; 3) saturation of $C_9$-$C_{10}$ double bond.

The two nitrogen atoms in YCK are in a very different steric environment. The indole moiety attached to $C_6$ is relatively free to rotate and its nitrogen is easily accessible, whereas the indole moiety forming part of the tetracycle is rigid and its nitrogen is shielded by the free indole at $C_6$. Thus, the introduction of a substituent to nitrogen invariably took place at the $C_6$-indole first. Acetylation, succinylation, and methylation of YCK gave the respective *N*-1'-substituted derivatives of yuehchukene, namely *N*-1'-acetyl-YCK (**2**), *N*-1'-succinyl-YCK (**3**), and *N*-1'-methyl-YCK (**4**). By methylation under more vigorous conditions, disubstituted *N*-1',*N*-5-dimethyl-YCK (**5**) could be obtained.

The *N*-1',*N*-5-dimethoxy-YCK (**6**) was prepared using a procedure analogous to our reported biomimetic synthesis of YCK [3] by employing *N*-methoxyindole (**14**) as the starting material. Thus *N*-methoxyindole [6] was converted to its aldehyde (**15**) by the Vilsmeier reaction [7]. In a typical Grignard reaction, treatment of the aldehyde (**15**) with 2-methylprop-2-enyl magnesium chloride in THF gave the alcohol (**16**) which upon dehydration with mesyl chloride in triethylamine gave the diene (**17**). The crude diene (**17**), without purification, was subjected to acid-catalysed dimerization in the presence of silica gel whereby *N*-1',*N*-dimethoxy-YCK (**6**) was isolated pure (scheme 1).

Similarly, 2,5'-dimethyl-YCK (**7**) and 2,5'-dimethoxy-YCK (**8**) were prepared from 5-methylindole (**18**) and 5-methoxy-indole (**19**) respectively (scheme 2). However, in these cases, an extra step to introduce a *N*-tosyl protecting group was necessary before the Grignard reaction. Removal of the *N*-tosyl protecting group occurred concomitantly with dehydration by treating the alcohol (**24**, **25**) with 3 N ethanolic potassium hydroxide.

Saturation of $C_9$-$C_{10}$ double bond was achieved by catalytic hydrogenation of YCK over Adam catalyst




**Scheme 1.** Reagents: i, $POCl_3$, DMF; ii, $CH_2$ = $CMeCH_2$-MgCl, THF; iii, Et3N, $MeSO_2Cl$; iv, MeONa, MeOH; v, silica gel, benzene 60°C.

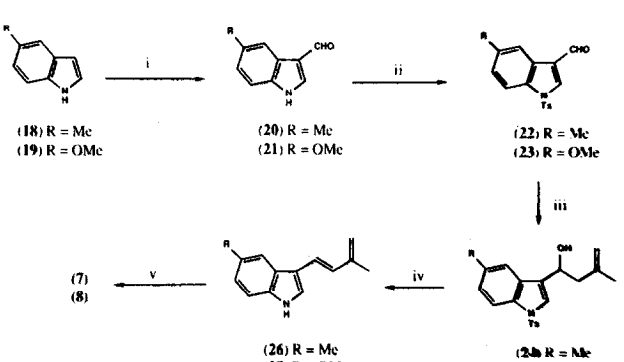
to give the 9,10-dihydro-YCK (**9**). Hydroxylation of $C_9$-$C_{10}$ double bond with osmium tetroxide resulted in the *cis*-addition of hydroxy groups from the less hindered β-side leading to the formation of 9,10-dihydroxy-YCK (**10**).

The 2,5'-dihydroyx-9,10-dihydro-YCK (**11**) was prepared from 2,5'-dimethoxy-YCK (8) by first saturating the $C_9$-$C_{10}$ double bond by catalytic hydrogenation, followed by cleaving the methyl ether (12) with ethanethiol in $AlCl_3$ and aqueous HCl [8]. Attempts to demethylate 2,5'-dimethoxy-YCK were not successful unless the $C_9$-$C_{10}$ double bond was saturated.

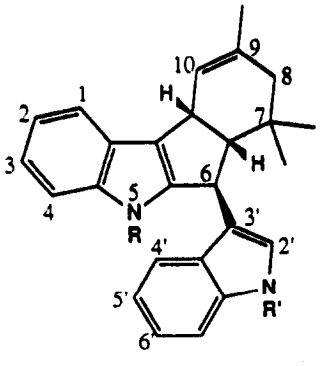
## Bioassay

*Anti-implantation activity*

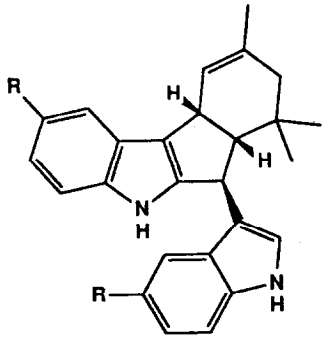
Eight week-old female Sprague Dawley rats, 200–220 g, were supplied by the University Animal House



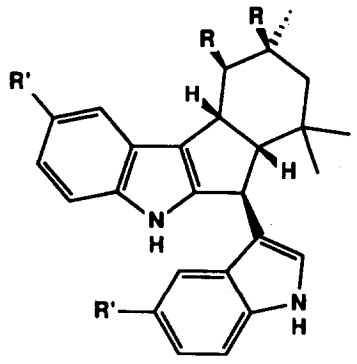

**Scheme 2.** Reagents: i, $POCl_3$, DMF; ii, NaH, TsCl, DME; iii, $CH_2$ = $CMeCH_2MgCl$, THF; iv, 3N KOH/EtOH; v, $CF_3COOH$, silica gel benzene, 60°C.



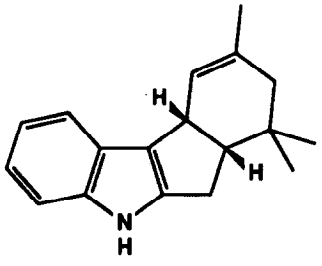
(1) R = H, R' = H
(2) R = H, R' = Ac
(3) R = H, R' = $COCH_2CH_2COOH$
(4) R = H, R' = Me
(5) R = Me, R' = Me
(6) R = OMe, R' = OMe



(7) R = Me
(8) R = OMe



(9) R = H, R' = H
(10) R = OH, R' = H
(11) R = H, R' = OH
(12) R = H, R' = OMe



(13)

on the morning when evidence of mating was indicated by the presence of post-coital plug. This was designated as day 1 of pregnancy ($PD_1$). The animals were then dosed orally in the morning with 1 ml of drug solution or vehicle from $PD_{1-4}$, or otherwise assigned. They were autopsied on $PD_8$. The number of pregnant animals was recorded. The uteri were removed, trimmed and photographed, then preserved in Bouin fixative. The absence of implantation sites was an indication of anti-implantation activity. All analogues were dosed to achieve an 'all or none' end point. Reduction in embryo size was rarely observed.

17α-Ethynylestradiol was used as a positive control (fig 1). Anti-implantation of analogues **2–11** is shown in table I.

*Estrogenic activity*

21–23 day-old female Sprague Dawley rats were ovariectomised and were allowed to recover for 3–7 d. After that they were dosed orally twice daily for 3 d with 0.2 ml of drug solution for each dose. A vaginal smear was made the day after the last dose and vaginal patency was noted. During autopsy the uterine horns were excised, trimmed free of fatty tissue and weighed. The increase in uterine weight was a measure of uterotrophic response to estrogenic activity.

*Drug formulation*

To formulate 10 ml of drug solution, for example, 1 ml EtOH was added to the weighed drug with vigorous stirring to achieve complete dissolution. One ml of benzyl benzoate was then added, followed by sufficient olive oil to a final vol of 10 ml and the whole preparation was mixed thoroughly.

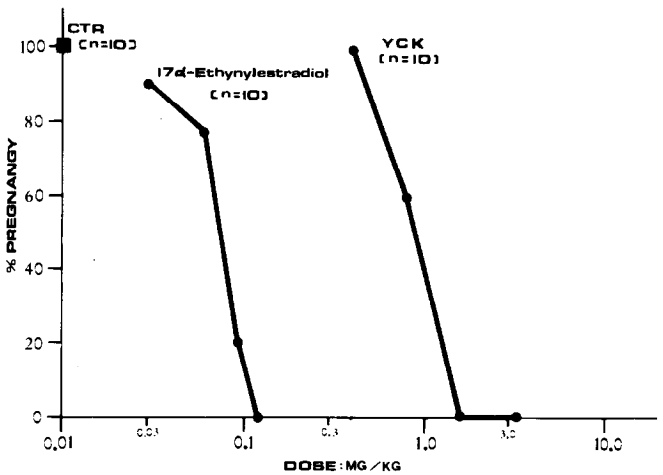



**Fig 1.** Dose response curve of YCK in anti-implantation assays in rats.

## Structure–function relationship

*Anti-implantation activity*

YCK was 100% active at 2.5 mg/kg, dosing $PD_{1-4}$. At this dose level, it was equally active by dosing on $PD_2$ only. Pure YCK (> 95%) had a minimal effective dose of 1.25 mg/kg. Although all YCK derivatives tested were at least > 85% pure as judged by HPLC profiles, their bioavailability may differ. Thus the optimal effective dose (oed) of YCK was set at 2.5 mg/kg, dosing $PD_{1-4}$, for the purpose of comparing the relative potency of different derivatives.

*N*-1'-Acetyl-YCK (**2**) was readily crystallizable but devoid of activity at 2 x oed (table I). By the same token, *N*-1'-succinyl-YCK (**3**) was not active at all at oed level. This derivative was water soluble at 1.2 mg/ml. Its water solubility should not affect its activity as YCK formulated as a fine suspension in 5% gelatin water had the same activity as YCK formulated in oil (unpublished results).

*N*-1'-Methylation of free indole moiety in YCK permitted the methylated derivative (**4**) to retain 30% of its potency. It was active at 3 x oed. However, methylation on the nitrogen atom of both indoles (**5**) totally abolished activity even at 6 x oed. By the same token, methoxylation of the nitrogen atom in both indoles (**6**) also abolished activity at 3 x oed.

Substitution on the benzene ring led to the disappearance of activity. Both 2,5'-dimethyl-YCK (**7**) and 2,5'-dimethoxy-YCK (**8**) were not active at 2 x oed and 4 x oed respectively.

Saturation of the $C_9$-$C_{10}$ double bond in 9,10-dihydro-YCK (**9**) did not affect activity at all. As a matter of fact, (**9**) was equipotent with the parent compound when tested concurrently over a wide range of doses. While this result suggests that $C_9$-$C_{10}$ double bond is indifferential to the expression of anti-implantation activity, 9,10-dihydroxy-YCK (**10**) was not active at 4 x oed.

Hydroxylation on the benzene rings also abolished activity. While 9,10-dihydro-YCK was equipotent, 2,5'-dihydroxy-9,10-dihydro-YCK (**11**) was not active at 1.25 mg/kg. Although this dose was only 0.5 x oed, YCK (95% pure) tested at the same dose level in the same experiment was 100% active.

*Estrogenic activity*

When 17α-ethynylestradiol was used as a positive control in anti-implantation assay, it showed a dose response curve parallel to YCK reaching 100% activity at 0.12 mg/kg and 1.6 mg/kg respectively. At 50% maximal activity, YCK carried 8.2% estrogenicity in anti-implantation assay (fig 1). Dosing at $PD_{1-3}$ only gave the same relative estrogenic potency.

When 17α-ethynylestradiol was used as a positive control in uterotrophic assay, it showed a linear dose response curve reaching a plateau at 0.03 mg/kg. The

**Table I.** Anti-implantation activity of substituted Yuehchukene (YCK) derivatives **2–11**.

| | | | *Dose (mg/kg, $PD_{1-4}$)* | *No preg No tested* | *Remarks* |
|---|---|---|---|---|---|
| I. N-substitution | | | | | |
| **2** | 5-H | 1'-Ac | 6.0 | 5/5 | –ve |
| **3** | 5-H | 1'-Suc | 2.4 | 5/5 | –ve |
| **4** | 5-H | 1'-Me | 7.5 | 1/5 | +ve |
| **5** | 5-Me | 1'-Me | 15.0 | 5/5 | –ve |
| **6** | 5-OMe | 1'-OMe | 7.5 | 5/5 | –ve |
| II. Aromatic substitution | | | | | |
| **7** | 2-Me | 5'-Me | 5.0 | 5/5 | –ve |
| **8**[a] | 2-OMe | 5'-OMe | 9.1 | 5/5 | –ve |
| III. $C_9$-$C_{10}$ saturation | | | | | |
| **9**[b] | 9-H,Me | 10-H,H | 3.0 | 0/8 | +ve |
| **10** | 9-OH,Me | 10-OH,H | 10.0 | 5/5 | –ve |
| IV. Aromatic hydroxylation | | | | | |
| **11** | 2-OH<br>9-H,Me | 5'-OH<br>10-H,H | 1.25 | 5/5 | –ve |
| V. Yuehchukene[c] | | | | | |
| **1** | YCK | | 1.25 | 0/5 | +ve |
| VI. Vehicle control[d] | | | 1 ml | 5/5 | –ve |

[a]Dosed $PD_{2-3}$ only; [b]also tested at 5 mg/kg with 100% activity (0/10); [c]YCK of 95% purity was tested in the same experiment as 11; [d]control groups from all experiments in table I had 100% fertility.

dose response curve of YCK followed a slower rising slope in a less parallel manner, reaching a plateau at 0.3 mg/kg which was 2/3 of the maximal response by estradiol. At 50% maximal activity, YCK carried 4.5% estrogenicity in uterotrophic assay. This result holds true in several experiments using YCK from different batches (fig 2). 9,10-Dihydro-YCK (**9**) which was equipotent in anti-implantation was also uterotrophic but soon reaching a plateau at 0.1 mg/kg at 50.6% increase of uterine weight. *N*-1',*N*-5-dimethyl-YCK (**5**) and 2,5'-dimethyl-YCK (**7**) were not active even at 3 mg/kg and 5 mg/kg respectively. However, *N*-1'-methyl-YCK (**4**) was active at 1 mg/kg. At this dose level, it could increase uterine weight by 50.9% and positive response only began at this dose level.

## Discussion

Yuehchukene (YCK) is a novel anti-implantation agent in rats not only for having a unique indeno-indole tetracyclic structure, but also for being an estrogenic compound without an oxygen function. Non-steroidal estrogenic compounds such as diethylstilbestrol, ORF-4563 [9], ORF-8511 [10], 2,3-diphenyl-indole derivatives [11] and coronaridine [12], all carried oxygen in their native structure. Therefore, it was assumed that YCK would become estrogenic after hydroxylation on the benzene ring during hepatic passage. Since methyl or methoxy substitution on the benzene ring rendered the corresponding YCK derivatives (**7**, **8**) inactive, this may imply that substitution on the 5-position of the indole aromatic centre prevents the formation of an active YCK derivative which is hydroxylated at this position. The fact that 2,5'-dihydroxy-9,10-dihydro-YCK (**11**) was inactive, or at least not as active as the parent compound, does not seem to support this hypothesis. Hydroxylation on 2,5'-position would bring YCK close to estrogen in structural resemblance. Indeed, if YCK was active by virtue of its estrogenicity, (**11**) should be more potent than YCK in anti-implantation, but this is not the case. It will be necessary to look for a new mechanism of anti-implantation (in rats).

Since *N*-1'-methylation could be tolerated at reduced potency but not *N*-1'-acetylation or *N*-1'-succinylation, a more bulky group substituted at *N*-1'-position would restrict the rotation of the free indole. It suggests that the relative position or planarity of the aromatic centres may be crucial to the expression of the biological activity as the *N*-1'-substituted derivative would have less chance to attain an optimal active conformation. It also suggests that the lone pair of electrons on the nitrogen atom must be freely accessible in order to express anti-implantation activity. Double substitution on both nitrogen atoms was not active at the same dose level or even twice as high.

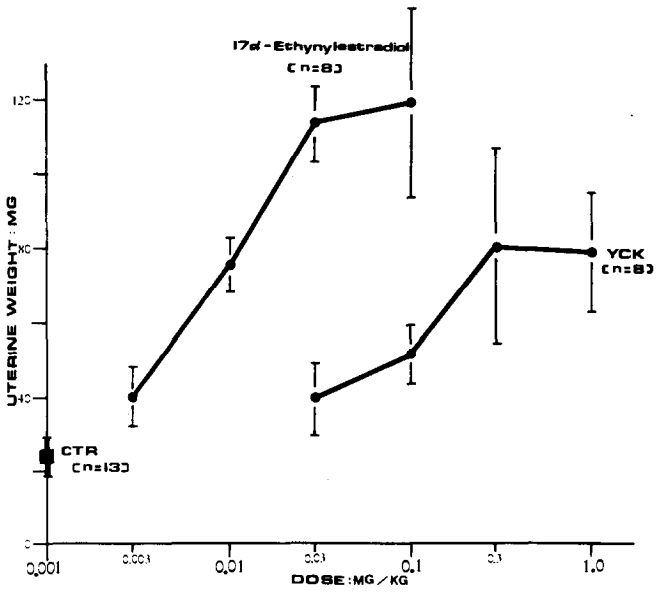



**Fig 2.** Dose response curve of YCK in uterotrophic activity in immature rats.

The present results indicate that where YCK or its derivative is active in anti-implantation, it is also uterotrophic. The dose response curves of YCK and estradiol in anti-implantation assay are entirely parallel. It suggests that YCK is a weak estrogen. However, this is not the case in uterotrophic assay. Here a lesser slope and a lower plateau in YCK indicated that it is at best a partial estrogen. If indeed YCK blocks implantation in rats by virtue of its estrogenicity acquired after metabolic transformation, it may not be expressed through the same structural basis as required in uterotrophic response. Hydroxylation on the $C_9$-$C_{10}$ double bond (**10**) could render the parent compound similar to an estrogenic metabolite. Further hydroxylation on the benzene ring would make YCK resemble estriol in structure. But the fact that both **10** and **11** were inactive (the estrogenic metabolites), if indeed YCK must be metabolised to become estrogenic, it is unlikely that it would be a hydroxylated derivative.

## Experimental protocols

Melting points were measured on a Reichert Kofler-hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 577 spectrophotometer and calibrated with polystyrene. NMR spectra were recorded on a Jeol FX-90Q spectrometer in deuteriochloroform unless otherwise stated with tetramethyl-silane as internal standard. Mass spectra were recorded on a Hitachi RMS-4 and VG 70-70F high resolution mass spectrometers. Flash chromatography used Kieselgel 60 (Merck) as the stationary phase [13]. Analytical HPLC was performed on a Beckmann Model 331 HPLC System with a

UV detector. Light petroleum refers to 40–60°C fraction which was redistilled before use. All reactions requiring anhydrous conditions used dry solvents and were conducted in apparatus dried in an oven at 120°C and under a static atmosphere of dry nitrogen. New compounds whose elemental compositions were established through accurate mass determination were shown to be homogeneous by spectroscopic and chromatographic methods. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. All compounds described are racemic.

*N-1'-Acetyl-yuehchukene* **2**
YCK (24 mg) was acetylated (acetic anhydride, 3 ml; anhydrous sodium acetate, 10 mg, reflux 4 h) to give **2** as a colourless solid (21 mg, 79%), mp = 243°C (from absolute ethanol) after silica gel chromatography; $\delta_H$ (90 MHz) 0.89 (3H, s, 7-Me), 1.12 (3H, s, 7-Me), 1.65 (1H, d, $J$ = 17 Hz, 8-H), 1.66 (3H, s, 9-Me), 2.25 (1H, d, $J$ = 17 Hz, 8-H), 2.56 (3H, s, COMe), 3.17 (1H, m, 6a-H), 4.01 (1H, m, 6-H), 4.53 (1H, d, $J$ = 8 Hz, 10a-H), 5.69 (1H, br s, 10-H), 7.51 (1H, br s, exchangeable with $D_2O$, NH), 7.59–7.09 (8H, m, ArH), and 8.44 (1H, d, $J$ = 6 Hz, 7'-H); *m/z* 408 ($M^+$).

*N-1'-Succinyl-yuehchukene* **3**
NaH (100 mg) was added to YCK (100 mg) in anhydrous THF (10 ml) at 0°C under nitrogen and was stirred for 5 h. Succinic anhydride was added and stirred for another 15 h. After adding dil HCl (1 ml), the solution was extracted with ether. Removal of solvent and chromatography of the residue on silica gel afforded **3** as a yellow solid (25 mg, 20%), mp = 146–148°C (from petroleum ether), $\delta_H$ (90 MHz) 0.84 (3H, s), 1.07 (3H, s), 1.64 (3H, s), 3.28–2.7 (5H, m), 4.0 (1H, m), 4.5 (1H, d), 5.64 (1H, br s), 7.5–7.0 (9H, m), 8.32 (1H, d, $J$ = 9 Hz); *m/z* 466 ($M^+$).

*N-1'-Methyl-yuehchukene* **4**
YCK (32 mg, 0.09 mmol) in THF (5 ml) was added dropwise to NaH (2.5 mg, 0.1 mmol) in anhydrous THF (5 ml) at room temperature under nitrogen. After stirring the mixture for 0.5 h, the whole was poured into ice water then extracted with ether. Removal of solvent and chromatography of the residue on silica gel yielded **4** (8 mg, 24%) as a light brown powder (found: $M^+$, 380.2257. $C_{27}H_{28}N_2$ requires M, 380.2246), mp = 127–129°C (from petroleum ether); $\nu_{max}$ 3400, 2900, 1610, 1460, 1450, 1370, 1365, 1330, 1250, 1150, 1130, 1120, 1110 and 740 $cm^{-1}$; δH (90 MHz), 0.84 (3H, s, 7-Me), 1.08 (3H, s, 7-Me), 1.65 (3H, s, 9-Me), 2.17 (1H, br s, 8-H), 2.35 (1H, br s, 8-H), 3.10 (1H, dd, $J$ = 7.5, 8.3 Hz, 6a-H), 3.68 (3H, s, N'-Me), 3.97 (1H, m, 10a-H), 4.50 (1H, d, $J$ = 8.3 Hz, 6-H), 5.68 (1H, br s, 10-H), 6.80–7.59 (10H, m, Ar-H).

*N-1',N-5-Dimethyl-yuehchukene* **5**
KH (35% in oil dispersion, 80 mg) was added to YCK (183 mg) in THF (5 ml) at room temperature. The wole was stirred until gas ceased to evolve. Methyl iodide (300 mg) was added. After stirring the mixture for another 0.5 h, water was added and then extracted with ether. Removal of solvent afforded compound **5** as a pale yellow solid (93 mg, 47%) (found: $M^+$, 394.2412. $C_{28}H_{30}N_2$ requires M, 394.2407); mp = 207–209°C (from petroleum ether); $\nu_{max}$ 3040, 2900, 1610, 1465, 1410, 1010 and 835 $cm^{-1}$; $\delta_H$ (90 MHz) 0.83 (3H, s, 7-Me), 1.08 (3H, s, 7-Me), 1.63 (1H, d, $J$ = 7.66 Hz, 8-H) 1.65 (3H, br s, 9-Me), 2.22 (1H, d, $J$ = 7.66 Hz, 8-H), 3.04 (1H, m, 6a-H), 3.10 (3H, s, NMe), 3.74 (3H, s, NMe), 4.03 (1H, m, 10a-H), 4.57 (1H, d, $J$ = 8.75 Hz, 6-H), 5.71 (1H, m, br s, 10-H), 6.87 (1H, s, 2'-H'), 7.63–6.89 (8H, m, Ar-H).

*1-Methoxyindole-3-carboxaldehyde* **15**
To a stirred, ice-cooled solution of anhydrous *N,N*-dimethylformamide (DMF) (12 ml of phosphoryl chloride (4.82 ml, 50 mmol) was added dropwise. 1-Methoxyindole **14** (5 g, 34 mmol) in DMF (3.75 ml) was then added over a period of 1 h. The mixture was kept at 45°C for another 1 h and poured into ice-water (78 ml). The solution was extracted with ether; the organic extracts were discarded. The aqueous layer was treated with NaOH (7.0 g) in water (40 ml) and extracted with ether. The combined extract was washed with brine, dried, and concentrated to give **15** as a brown oil (4.76 g, 80%), $\nu_{max}$ 3120, 2950, 2820, 2720, 1660, 1510, 1365, 1310, 1230, 1020 and 930 $cm^{-1}$; $\delta_H$ (90 MHz) 4.00 (3H, s, OMe), 7.19–7.36 (3H, m, 5-H, 6-H and 7-H), 7.79 (1H, s, 2-H), 8.29 (1H, m, 4-H) and 9.81 (1H, s, CHO); $\delta_C$ (22.5 MHz) 66.14 (OMe), 108.29 (C-7), 113.38 (C-3), 120.97 ((C-3a), 121.34 (C-4), 122.81 (C-5), 123.95 (C-6), 131.91 (C-2), 132.18 (C-7a) and 183.59 (CHO); *m/z* 175 ($M^+$); anal $C_{10}H_9NO_2$ (C, H, N).

*3-(1'-Hydroxy-3'-methylbut-3'-enyl)-1-methoxyindole* **16**
Magnesium turnings (0.368 g, 16 mmol), 3-chloro-2-methylpropene (0.3 ml) and a few crystals of iodine in anhydrous THF (3 ml) were stirred vigorously under agon atmosphere. When the reaction set in, the aldehyde **15** (1.13 g, 6.4 mmol) and 3-chloro-2-methylpropene (1.6 ml, 16 mmol) in THF (20 ml) was added dropwise so as to maintain a gentle reflux. After complete addition, it was refluxed for 45 min, cooled and poured into saturated ammonium chloride solution. The aqueous solution was extracted with ether and concentrated to give the alcohol **16** as a brown liquid (1.43 g, 96%); $\lambda_{max}$ (EtOH) 220 (log ε 4.47), 274 (3.71), 287 (3.71) and 298 nm (3.63); $\nu_{max}$ 3420, 3080, 2950, 1650, 1540, 1445, 1080, 1040, 880 and 715 $cm^{-1}$; $\delta_H$ (90 MHz) 1.71 (3H, s, 3'-Me), 2.53 (2H, d, $J$ = 7.0 Hz, 2'-H2), 2.64 (1H, br s, exchangeable with D2O, OH), 3.87 (3H, s, OMe), 4.78 (2H, m, 4'-H2), 5.01 (1H, t, $J$ = 7.0 Hz, 1'-H), 6.94–7.69 (4H, m, 4-, 5-, 6- and 7-H) and 7.10 (1H, s, 2-H); $\delta_C$ (22.5 MHz) 22.48 (3'-Me), 46.48 ($CH_2$), 65.39 (OMe), 65.82 (CH), 108.40 (C-7), 113.22 (C-4'), 115.45 (C-3), 119.89 (C-4), 120.21 (C-2 and C-5), 122.32 (C-3a), 122.54 (C-6), 132.73 (C-7a) and 142.64 (C-3'); *m/z* 231 ($M^+$); anal $C_{14}H_{17}NO_2$ (C, H, N).

*1-Methoxy-3-(3'-methylbuta-1',3'-dienyl)-indole* **17**
To a solution of the alcohol **16** (3 g, 16 mmol) in anhydrous THF (56 ml) stirred under argon at –60°C was added triethylamine (3.1 ml, 22 mmol) and methanesulphonyl chloride (1.2 ml, 15.6 mmol). The resulting solution was warmed to rt in 1.5 h and a white solid formed during this period. Sodium methoxide freshly prepared from dissolving sodium (0.45 g) in anhydrous methanol (4 ml) was added. The mixture was refluxed genlty for 15 min. The precipitate was filtered off and the filtrate was concentrated to afford the diene **17** as a light brown liquid (1.04 g, 37.5%); $\nu_{max}$ 3130, 2980, 2940, 1630, 1445, 930, 850 and 710 $cm^{-1}$; $\delta_H$ (90 MHz) 1.96 (3H, s, Me), 3.89 (3H, s, OMe), 4.99 (2H, m, 3'-CH2), 6.60 and 6.90 (2H, ABq, $J$ = 16.19 Hz, *trans* CH = CH), 7.04–7.41 (4H, m, ArH) and 7.84 (1H, m, 7-H); $\delta_C$ (22.5 MHz) 18.47 (Me), 65.66 (OMe), 108.51 (C-7), 111.22 (C-3), 114.85 (C-4'), 120.16 (C-4), 120.54 (C-2), 120.70 (C-5), 121.51 (C-2'), 122.49 (C-3a), 122.92 (C-6), 129.37 (C-1'), 130.89 (C-7a) and 142.48 (C-3').

The diene **17** was too labile for further purification and was used directly for subsequent reactions.

*N-1',N-5-Dimethoxy-yuehchukene* **6**
To a suspension of silica gel (15 g) in benzene (50 ml) at rt under nitrogen the diene **17** (1 g, 50 mmol) and trifluoroacetic

acid in benzene (0.2 mmol $ml^{-1}$; 0.8 ml) were added. The whole was stirred at 80°C for 1 h, cooled, and filtered. The silica gel was washed with diethyl ether. The combined organic extract was dried and evaporated to give a dark brown viscous oil which upon purification by a Lobar silica gel column (Si60, 40–60 μm) eluted with ether-light petroleum 4/7) yielded **6** as a solid (110 mg, 11%) (found: $M^+$, 426.2301. $C_{28}H_{30}N_2O_2$ requires M, 426.2305 mp = 146–147°C; $\nu_{max}$ 3413, 2930, 1671, 1449, 1093, 955 and 825 $cm^{-1}$; $\delta_H$ (90 MHz), 0.84 (3H, s, 7-Me), 1.10 (3H, s, 7-Me), 1.64 (3H, s, 9-Me), 3.12 (1H, dd, *J* = 7, 7 Hz, 6a-H), 3.27 (3H, s, OMe), 3.81 (1H, m, 10a-H), 4.03 (3H, s, OMe), 4.65 (1H, d, *J* = 7 Hz, 6-H), 5.69 (1H, m, 10-H), 7.01–7.58 (9H, m, Ar-H).

*5-Methyl-*N*-tosylindole-3-carboxaldehyde **22***
To a suspension of sodium hydride (50% oil dispersions; 4 g, 83 mmol) in anhydrous dimethoxyethane (DME) (10 ml) was added a suspension of 5-methylindole-3-carboxaldehyde **14** (10 g, 63 mmol) in anhydrous DME. The mixture was warmed to 60°C for 1 h, cooled, and filtered. The filtrate was poured into excess cold sodium hydrogen carbonate solution. The brown precipitate formed was collected. Recrystallization from chloroform afforded **22** as a light brown solid (17.5 g, 89%) (found: $M^+$, 313.0764. $C_{17}H_{15}NO_3S$ requires M, 313.0769), mp = 167–168°C; $\nu_{max}$ 3120, 1600, 1450, 1375, 1170 and 820 $cm^{-1}$; $\delta_H$ (90 MHz) 2.36 (3H, s, 5-Me), 2.43 (3H, s, Ar'Me), 7.27 (2H, d, *J* = 7.9 Hz, 3'- and 5'-H), 7.80–7.21 (2H, m, 2- and 6-H), 7.83 (2H, d, *J* = 7.9 Hz, 2'- and 6'-H), 8.05 (1H, br s, 7-H), 8.17 (1H, s, 4-H) and 10.06 (1H, s, CHO).

*5-Methoxy-*N*-tosylindole-3-carboxaldehyde **23***
*N*-tosylation of compound **21** by a method similar to the preparation of **22** afforded the aldehyde **23** as a solid (80%) mp = 128–129°C. (Found: $M^+$, 329.0719. $C_{17}H_{15}NO_4S$ requires M, 329.0718); $\nu_{max}$ 3115, 2920, 2810, 1678, 1601, 1460, 1375, 1170 and 820 $cm^{-1}$; $\delta_H$ (90MHz) 2.37 (3H, s, ArMe), 3.84 (3H, s, OMe), 6.98–8.17 (8H, ArH) and 10.06 (1H, s, CHO).

*5-Methyl-*N*-tosyl-3-(1-hydroxy-3-methyl-but-3-enyl)indole **24***
The alcohol **24** was prepared by a procedure similar to that for the preparation of **16** above. From the aldehyde **22** (7 g), the desired alcohol **24** was obtained as a pale green viscous oil (3.86 g, 48%) (found: $M^+$, 369.1390. $C_{21}H_{23}NO_3S$ requires M, 369.1393); $\nu_{max}$ 3550, 2920, 1600, 1450, 1370, 1170, 895 and 810 $cm^{-1}$; $\delta_H$ (250 MHz) 1.81 (3H, s, 3'-Me), 2.2 (1H, br s, OH), 2.35 (3H, s, 5-Me), 2.41 (3H, s, Ar-Me), 2.6 (2H, d, *J* = 5.3 Hz, 2'-$H_2$), 4.85–4.94 (2H, 2 x s, 4'-$H_2$), 5.14 (1H, t, *J* = 5.3 Hz, 1'-H) and 7.13–7.84 (8H, m, Ar-H).

*5-Methoxy-*N*-tosyl-3-(1'-hydroxy-3'-methylbut-3'-enyl)indole **25***
The alcohol **25** was prepared from the aldehyde **23** by a method similar to the preparation of **16**. Compound **25** was obtained as an oil (50%); $\nu_{max}$ 3600–3800, 2320, 1590, 1370, 895 and 810 $cm^{-1}$; $\delta_H$ (90MHz) 1.77 (3H, s, 3'-Me), 2.30 (3H, s, Ar-Me), 2.25 (1H, s, OH), 2.56 (2H, d *J* = 6.6 Hz, 2'-$H_2$), 3.79 (3H, s, OMe), 4.81–4.90 (3H, m- 1'- and 4'-$H_2$) and 6.90–7.85 (8H, m, Ar-H); anal $C_{21}H_{23}NO_4S$ (C, H, N).

*5-Methyl-3-(3'-methylbuta-1',3'-dienyl)-indole **26***
To a solution of the alcohol **24** (2.5 g, 68 mmol) in absolute ethanol (10 ml) was added 3 N alcoholic KOH water/ethanol, 1/4; 100 ml) was added. After stirring for 2 h at 60°C the mixture was cooled, diluted with ice water, and extracted with ether. The extract was washed with awter and dried. Removal of the solvent and chromatography of the residue on silica gel afforded the diene **26** (0.77 g, 59%) (found: $M^+$, 197.1163. $C_{14}H_{15}N$ requires M, 197.1166); $\nu_{max}$ 3414, 1624, 1585, 1261, 1172, 919 and 886 $cm^{-1}$; $\delta_H$ (90 MHz, $C_6D_6$), 1.85 (3H, s, 3'-Me), 2.24 (3H, s, 5-Me), 5.05 (1H, br s, 4'-H), 5.23 (1H, s, 4'-H), 6.72–7.68 (7H, m, 1'-, 2'- and Ar-H).

*5-Methoxy-3-(3'-methylbuta-1',3'-dienyl)-indole **27***
The diene **27** was prepared from the alcohol **23** by a method similar to the preparation of **26**. Compound **27** was obtained as an oil in 50% yield (found: $M^+$, 213.1156. $C_{14}H_{15}NO$ requires M, 213.1150); $\nu_{max}$ 3385, 1318, 1245, 1089, 1034, 882 and 795 $cm^{-1}$; $\delta_H$ (90 MHz, $C_6D_6$) 1.98 (3H, s, 3'-Me), 3.49 (3H, s, OMe), 5.02 (1H, s, 4'-H), 5.12 (1H, s, 4'-H) and 6.67–7.53 (7H, m, 1'-, 2' and Ar-H).

*2,5'Dimethyl-yuehchukene **7***
The diene **26** (1 g) was subjected to dimerization under conditions similar to the preparation of **6** whereby **7** was obtained as a white powder (80 mg, 8%), mp = 129–131°C (from petroleum ether) (found: $M^+$, 294.2404. $C_{28}H_{30}N_2$ requires M, 394.2402); $\nu_{max}$ 3410, 2950, 2920, 2860, 1590, 1425, 1100 and 800 $cm^{-1}$; $\delta_H$ (250 MHz) 0.84 (3H, s, 7-Me), 1.11 (3H, s, 7-Me), 1.57 (1H, d, *J* = 16 Hz, 8-H), 2.23 (1H, d, *J* = 16 Hz, 8-H), 1.63 (3H, s, 9-Me), 2.38 (3H, s, 2-Me), 2.43 (3H, s, 5'-Me), 3.11 (1H, dd, *J* = 8.1, 7 Hz, 6a-H), 3.98 (1H, m, 10a-H), 4.44 (1H, d, J = 8.1 Hz, 6-H), 5.64 (1H, br s, 10-H), 6.85–7.01 (4H, m, Ar-H) and 7.21–7.35 (4H, m, Ar-H); $\delta_C$ (22.5 MHz) 21.52, 21.57, 24.06, 28.94, 29.18, 33.46, 37.49, 38.23, 41.04, 60.47, 110.90, 111.28, 118.06, 118.11, 118.80, 119.79, 121.88, 122.34, 123.01, 123.76, 124.48, 127.06, 128.61, 128.71, 130.07, 134.81, 134.81, 138.49 and 145.52.

*2,5'-Dimethoxy-yuehchukene **8***
2,5'-Dimethoxy-YCK **8** was prepared from the crude diene **27** by a procedure similar to the preparation of **6**. The compound **8** thus obtained was a solid (9%), mp = 118–119°C (from petroleum ether) (found: $M^+$, 426.2311. $C_{28}H_{30}N_2O_2$ requires M, 426.2305); $\nu_{max}$ 3420, 3320, 2960, 2840, 1610, 1580, 1480, 1430, 1210 and 1180 $cm^{-1}$; $\delta_H$ (90 MHz, $C_6D_6$) 0.94 (3H, s, 7-Me), 1.15 (3H, s, 7-Me), 1.56 (1H, d, J = 14 Hz, 8-H), 1.65 (3H, s, 9-Me), 2.51 (1H, d, *J* = 14 Hz, 8-H), 3.16 (1H, t, *J* = 7.9 Hz, 6a-H), 3.39 (3H, s, OMe), 3.59 (3H, s, OMe), 4.10 (1H, m, 10a-H), 4.50 (1H, d, *J* = 7.9 Hz, 6-H), 5.85 (1H, br s, 10-H) and 6.46–7.23 (9H, m, Ar-H); $\delta_C$ (22.5 MHz) 24.09, 28.92, 29.33, 32.82, 37.10, 40.49, 44.93, 55.78, 56.03, 61.07, 101.10, 101.19, 109.98, 111.87, 112.09, 112.16, 118.26, 122.04, 122.77, 122.83, 124.54, 127.08, 130.29, 131.65, 135.47, 146.09, 153.92 and 154.02.

*9,10-Dihydro-yuehchukene **9***
YCK (80 mg) in 10 ml of benzene saturated with water (10 ml) was hydrogenated over Adam catalyst at rt under 40 psi pressure for 40 h. The mixture was filtered through celite to remove platinum black. Removal of the solvent afforded the hydrogenated product **9** as a brown oil (60 mg, 75%) (found: $M^+$, 368.2247. $C_{26}H_{28}N_2$ requires M, 368.2251); $\delta_H$ (90 MHz) 0.70 (3H, s, 7-Me), 0.81 (3H, d, *J* = 6.12 Hz, 9-Me), 0.97 (3H, s, 7-Me), 2.93 (1H, dd, *J* = 6.12, 10.06 Hz, 6a-H), 3.36 (1H, d, *J* = 10.06 Hz, 10a-H), 4.46 (AH, d, *J* = 10.06 Hz, 6-H), 6.84–7.03 (10H, m, NH and Ar-H) and 8.02 (1H, br s, NH); $\delta_C$ (22.5 MHz) 29.71, 29.76, 22.78, 31.06, 33.52, 34.18, 36.50, 38.32, 40.99, 45.74, 111.20, 111.57, 111.71, 118.43, 119.38, 119.42, 119.55, 120.29, 121.97, 122.35, 124.17, 126.86, 136.46, 139.75 and 145.23.

*9,10-Dihydroxy-yuehchukene **10***
To a solution of YCK (0.225 g, 0.68 mmol) and pyridine (0.109 ml, 1.36 mmol) in anhydrous ether stirred at –5°C, $OsO_4$

(0.17 g, 0.68 mmol) in ether (5 ml) was added dropwise. The whole was stirred for 2 h. Yellow solid formed was collected by filtration. It was redissolved in dichloromethane (30 ml), treated with 10% mannitol in aqueous NaOH (5%; 25 ml), and stirred at 0°C overnight. The aqueous solution was extracted with dichloromethane and dried. Removal of the solvent and chromatography of the residue on silica gel afforded **10** as a yellow solid (41 mg, 15%); (found: $M^+$, 400.2146. $C_{26}H_{28}N_2O_2$ requires M, 400.2144); mp = 159–161°C (from hexane); $\nu_{max}$ 3420, 2960, 2920, 1620, 1580 and 1460 $cm^{-1}$; $\delta_H$ (90 MHz), 0.80 (3H, s, 7-Me), 1.14 (3H, s, 7-Me), 1.36 (3H, s, 9-Me), 1.55 (2H, m, 2-, 8-H), 1.73 (1H, br s, exchangeable with $D_2O$, OH), 1.94 (1H, br s, exchangeable with $D_2O$, OH), 2.97 (1H, d, $J$ = 10.28 Hz, 6-H), 3.21 (1H, dd, $J$ = 10.06, 6.56 Hz, 10a-H), 3.62 (1H, dd, $J$ = 10.28, 6.45 Hz, 6a-H), 4.31 (1H, d, $J$ = 10.06 Hz, 10-H) and 6.42–7.86 (11H, m, Ar-H).

*2,5'-Dimethoxy-9,10-dihydro-yuehchukene* **12**

2,5'-Dimethoxy-YCK **8** (720 g) in benzene (30 ml) saturated with water was hydrogenated over Adam catalyst at rt under 40 psi pressure for 3 d. Removal of platinum black and evaporation of solvent gave a residue which upon chromatography afforded the hydrogenated compound **12** (650 mg, 90%); $\delta_H$ (90 MHz) 0.90 (3H, d, $J$ = 7 Hz, 9-Me), 0.92 (3H, s, 7-Me), 1.04 (3H, s, 7-Me), 1.42–2.21 (4H, m, 8- and 10-$H_2$), 3.00 (1H, dd, $J$ = 6.12, 9.8 Hz, 6a-H), 3.30 (1H, m, 10a-H), 3.36 (3H, s, OMe), 3.62 (3H, s, OMe), 4.43 (1H, d, $J$ = 9.8 Hz, 6-H), 6.48 (1H, d, $J$ = 2.62 Hz, 2'-H) and 6.62–7.45 (8H, m, NH and ArH); $m/z$ 428 ($M^+$).

*2,5'-Dihydroxy-9,10-dihydro-yuehchukene* **11**

To a solution of $AlCl_3$ (530 mg, 4 mmol) in ethanethiol at 0°C compound **12** (430 mg, 1 mmol) was added dropwise. After stirring for 0.5 h, the whole was poured into ice-water, acidified with dil hydrochloric adic, and extracted with chloroform. Evaporation and purification of the residue on silica gel column afforded **11** as a white solid (130 mg, 20%) (found: $M^+$, 400.2141. $C_{26}H_{28}N_2O_2$ requires M, 400.2144); mp = 132–134°C (from petroleum ether); $\delta_H$ (90 MHz) 0.85 (3H, s, 7-Me), 0.94 (3H, d, $J$ = 6.13 Hz, 9-Me), 1.00 (3H, s, 7-Me), 1.42–2.25 (4H, m, 8- and 10-$H_2$), 2.98 (1H, dd, $J$ = 3.5, 9.6 Hz, 6a-H), 3.30 (1H, dd, J = 3.5, 11.6 Hz, 10a-H), 3.74 (1H, br s, OH), 3.95 (1H, br s, OH), 4.22 (1H, d, $J$ = 9.6 Hz, 6-H), 6.22 (1H, br s, 2'-H) and 6.43–7.64 (8H, m, NH and ArH).

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