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Short communication

Synthesis and in vitro antibacterial activity of novel heterocyclic derivatives of 18-nor-equilenin

A.V. Karnik [a,*], N.J. Malviya [a], A.M. Kulkarni [a], B.L. Jadhav [b]

[a] *Department of Chemistry, University of Mumbai, Vidyanagari, Kalina, Mumbai 400098, India*
[b] *Department of Life Sciences, University of Mumbai, Vidyanagari, Kalina, Mumbai 400098, India*

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Abstract

Syntheses of novel heterocyclic derivatives of 18-nor-equilenin, namely, (12*H*-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**/**b**) and its fused [1,2,4]triazolo derivatives6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-a]phenanthrene (**5a**/**b**), 10-methyl-6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-a]phenanthrene (**6a**/**b**) and tetrazolo derivatives 1-substituted-6*H*-5-oxa-7-thia-8,9,10,10*a*-tetraaza-pentaleno [4,5-a]phenanthrene (**7a**/**b**) along with their antibacterial activities are reported.

Keywords: 1,2,3,4-Tetrazol; 1,3,4-Triazole; Heterocyclic analog of 18-nor-equilenin; Heterosteroid; Azasteroid; Thiasteroid; Oxasteroid and antibacterial activity

1. Introduction

Steroids are widely distributed in nature and its members display much diversity in molecular structure [1]. Heterosteroids comprise of molecules with structural features characteristic of steroids such as tetra-cyclic cyclopenta[*a*]phenanthrene framework constituting ABCD ring system, also compounds where additional rings are fused to the main tetra-cyclic framework [2]. Heteroatom/s can be a part of ABCD ring system or other fused ring/s. Heterosteroids are widely distributed in nature [3]. The heterosteroidal compounds containing various combinations of heteroatoms are reported to exhibit diverse bioactivities [4–11] and have led to the synthesis of many modified steroidal hormones, some of which are now in use as drugs [12,13]. Azasteroids are by far the most common of the heterocyclic steroids [13]. Incorporation of oxygen in some steroidal system has resulted in enhanced bioactivity [14] in many cases, while the introduction of sulfur instead of methylene group in the steroidal system brings about electronic and steric changes in the structure and was considered to play an important role in the properties of the molecules [14,15]. Due to development of large number of present antibiotic resistant bacteria's, the discovery and development of newer antibacterial compounds have got importance and many groups are working towards the development of novel antibacterial agents [16]. The present paper discusses the synthesis of 2-hydrazino heterocyclic derivatives of 18-nor-equilinin namely, 1-substituted-16-(12H-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**, **b**) and its [1,2,4]triazolo derivatives: 1-substituted-6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-a]phenanthrene (**5a**, **b**), 1-substituted-10-methyl-6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-a]phenanthrene (**6a**, **b**) and tetrazolo derivatives: 1-substituted-6*H*-5-oxa-7-thia-8,9,10,10*a*-tetraaza-pentaleno[4,5-a]phenanthrene (**7a**, **b**).

2. Chemistry

3-Bromonaphthopyran (**2a**) and its 3,7-dibromo derivative (**2b**) were found to be the right precursors for the synthesis of **4a**, **b** as they provided the ABC ring of the steroidal system. **4a**/**b** could be easily obtained in a high yield process by treatment of **2a**/**b** with thiosemicarbazides. The hydrazine moiety in **4a**/**b** was effectively used for the construction of triazolo and tatrazolo fused systems in **5a**/**b**, **6a**/**b** and **7a**/**b**, respectively. The reaction of formic acid with **4a**/**b** gave **5a**/**b** in 3 h with excellent yields (90–92%). The reaction of acetic anhydride

* Corresponding author.
E-mail address: avkarnik@hotmail.com (A.V. Karnik).

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with **4a**/**b** gave **6a**/**b** in 5 h with similar excellent yields (87–89%). Whereas reaction of **4a**/**b** with nitrous acid gave **7a**/**b** at r.t. in less than 1 h with equally excellent yields (89–91%).

3. Pharmacology

The in vitro antibacterial activity of these compounds, **4a**/**b**, **5a**/**b**, **6a**/**b** and **7a**/**b** (1000, 500, 250, 100, 50 μg ml^{-1}) were tested against gram-positive [*Staphylococcus aureus*, *Bacillus subtilis*] and gram-negative bacteria [*Escherichia coli*, *Pseudomonas aeruginosa*] which were clinical isolates from Hafkkins Laboratory, Mumbai (India). They were maintained on nutrient agar (Himedia Lab. Ltd., India) slants at 4 °C prior to use for antimicrobial susceptibility test. The bioassay of these compounds was carried out by agar cup method [17] against test microorganism. All these tests were carried out in triplicate and the average values for zone of inhibition in millimeters (mm) were taken after 24 h of incubation. The compounds were dissolved in methanol. Amoxicillin [18] and vancomycin [19] were used as positive control for both gram-positive gram-negative bacteria's. The zone of inhibition and minimum inhibitory concentration (MIC) were noted. The results of such studies are presented in Tables 1–3.

4. Results and discussion

The anti bacterial activity of compounds **4a**/**b**, **5a**/**b**, **6a**/**b** and **7a**/**b** were assessed in side-by-side comparison with amoxicillin and vancomycin against some gram-positive [*S. aureus*, *B. subtilis*] and gram-negative [*E. coli*, *P. aeruginosa*] bacteria using agar cup method [17] and results are summarized in Tables 1 and 2 while the result of MIC of standards have been reported in Table 3. The antibacterial activity data indicate that compounds **4a**/**b**, **5a**/**b**, **6a**/**b** and **7a**/**b** showed good activity against all the tested bacteria's. Compound **5b** was found to be most potent against all the organisms tested at MIC 50 μg ml^{-1} except *P. aeruginosa* where the MIC was found out to be 100 μg ml^{-1}. Compounds **5a** and **7b** were also found to be very potent as well. Synthesis of newer bioactive heterocycles have received greater imputes due to development of various present time drug resistant pathogens and discovery of various newer diseases [16]. Some of the desired characteristics of potential drug candidates are the feasibility of chemical synthesis at the gram level (and preferably at the kilogram level), and demonstrated potency as measured in in vitro cell culture assay [20]. The newer antibacterial agents which have structural similarity to eqilenin/nor-equilenin [13], with demon-

Table 1
Antibacterial screening data

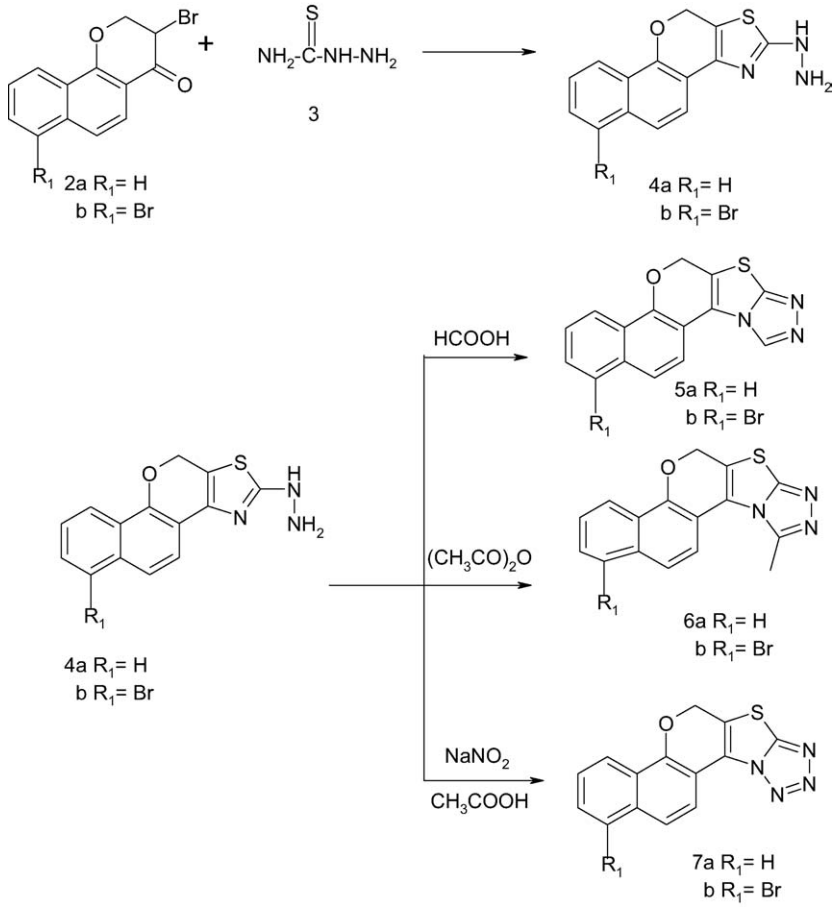
Serial number	Compound	Zone of inhibition (mm) activity at concentration of 1000 μg ml^{-1}			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
1	**4a**	15	14	18	09
2	**4b**	14	13	17	08
3	**5a**	18	15	18	11
4	**5b**	19	16	16	10
5	**6a**	16	12	15	09
6	**6b**	15	11	14	07
7	**7a**	14	14	12	06
8	**7b**	14	12	11	07
Standard	Amoxicillin	17	16	17	11
	Vancomycin	19	18	17	19

Table 2
Antibacterial screening data

Serial number	Compound	MIC in μg ml^{-1}			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
1	**4a**	500	250	500	500
2	**4b**	250	250	100	500
3	**5a**	100	50	50	100
4	**5b**	50	50	50	100
5	**6a**	250	100	250	500
6	**6b**	100	250	250	500
7	**7a**	250	250	100	500
8	**7b**	100	50	50	500

Table 3
Antibacterial screening data of standards used

Serial number	Compound	Zone of inhibition (mm) [MIC in μg ml^{-1}]			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Std 1	Amoxicillin	10 (100)	11 (100)	14 (100)	13 (100)
Std 2	Vancomycin	11 (50)	14 (50)	15 (50)	12 (50)



Scheme 1.

strated potency were successfully synthesized by practically simple and high yielding reactions (Scheme 1).

5. Experimental section

Melting points reported are uncorrected. IR spectra were recorded on a Shimadzu FTIR-4200 spectrometer and ^{1}H NMR spectra were recoded on Varian EM-360L (60 MHz) using TMS as an internal standard. Mass spectra were recorded on GC-MSQP-1000. Elemental analyses were carried on Carlo Enra EA-1108 elemental analyzer. TLC checked the homogeneity of compounds on silica gel.

2,3-Dihydro-4*H*-naphtho[1,2-*b*]pyran-4-one [21] (**1a**) and its 7-bromo-2,3-dihydro-4*H*-naphtho[1,2-*b*]pyran-4-one [22] (**1b**) derivative were synthesized as reported in the literature.

*5.1. Synthesis of 3-bromo-2,3-dihydro-4H-naphtho[1,2-b] pyran-4-one (**2a**) and 3,7-dibromo-2,3-dihydro-4H-naphtho [1,2-b]pyran-4-one (**2b**)*

A mixture containing 2,3-dihydro-4*H*-naphtho[1,2-*b*]pyran-4-one **1a** (1.96 g, 0.01 mol)/7-bromo-2,3-dihydro-4*H*-naphtho [1,2-*b*]pyran-4-one **1b** (2.77 g, 0.01 mol) and N-bromo succinimide (1.78 g, 0.01 mol) along with catalytic amount of benzoyl peroxide (20 mg) was refluxed in carbon tetrachloride (30 ml) for 4 h. The reaction mixture was then cooled and succinimide in the solid form was separated by filtration. The organic layer was then concentrated to 1/4th the original volume and after over night refrigeration compound **2a**/**2b** crystallized out from the reaction mixture.

*5.2. 3-Bromo-2,3-dihydro-4H-naphtho[1,2-b]pyran-4-one (**2a**) [21]*

Yield 2.63 (92%), m.p. 154 °C, IR (KBr): C=C 1560, 1600, C=O 1680 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.5(m, 3H, CH and CH_2) and 7.1–8.0 (m, 6H, Ar). Anal. Calcd. C 56.35, H 3.27, Br 28.83. Found C 56.00, H 2.98, Br 28.60.

*5.3. 3,7-Dibromo-2,3-dihydro-4H-naphtho[1,2-b]pyran-4-one (**2b**)*

Yield 3.41 g (96%), m.p. 220 °C, IR (KBr): C=C 1580, 1610, C=O 1685 cm^{-1}. ^{1}H NMR($CDCl_3$): δ 3.8(m, 3H, CH and CH_2) and 7.2–7.9 (m, 5H, Ar). Anal. Calcd. C 43.86 H 2.27, Br 44.89. Found C 43.50, H 1.98, Br 44.62.

*5.4. (2H-11-oxa-17-thia-15-aza-cyclopenta[a]phenanthrene-16-yl)-hydrazine (**4a**)*

3-Bromo-2,3-dihydro-4*H*-naphtho[1,2-*b*]pyran-4-one (**2a**) (2.77 g, 10 mmol) was heated with thiaosemicarbazide (**3**)

(0.91 g, 10 mmol) in 1,4-dioxane (10 ml) at 75–80 °C 4 h to form the hydrobromide salt of **4a** which precipitated out. The reaction mixture was then cooled and vacuum filtered. The solid was then stirred for 30 min with saturated solution of sodium bicarbonate. The solid was then filtered and heated with an activated animal charcoal (0.5 g) in ethanol (25 ml) for 20 min. The filtrate on cooling to room temperature gave pale yellow solid of (2*H*-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**). Yield 2.47 g (92%), m.p. 220 °C (dec.), IR (KBr) (C=C) 1580, NH_2 3200, 3250 and NH 3400 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.6 (s, 1H, NH), 1.9 (s, 2H, NH_2), 5.4 (s, 2H, CH_2) and 7.3–8.3 (m, 6H, Ar), MS spectrum showed *m/z* 269 (M^+), 270 $(M + 1)^+$ and 271 $(M + 2)^+$. Anal. Calcd. C 62.45, H 4.09, N 15.61, S 11.90. Found C 62.25, H 3.89, N 15.41, S 11.62.

*5.5. 4-Bromo-(12H-11-oxa-17-thia-15-aza-cyclopenta[a] phenanthrene-16-yl)-hydrazine (**4b**)*

Yield 3.33 g (96%) m.p. 241 °C (dec.), IR (KBr) 1580, 1620 NH_2 3150, 3200 and NH 3300 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.7 (s, 1H, NH), 1.9 (s, 2H, NH_2), 5.3 (s, 2H, CH_2) and 7.2–8.1 (m, 5H, Ar). Anal. Calcd. C 48.28, H 2.87, Br 22.99, N 12.07, S 9.20. Found C 48.00, H 2.61, Br 22.72, N 11.80, S 9.00.

*5.6. 6H-5-oxa-7-thia-8,9,10a-triaza-pentaleno[4,5-a] phenanthrene (**5a**)*

(12*H*-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**) (2.69 g, 10 mmol) was refluxed with formic acid (25 ml) for 3 h. The reaction mixture was then cooled and neutralized with saturated solution of sodium bicarbonate. The solid obtained was then boiled with 0.5 g of activated animal charcoal and ethanol (50 ml) for 30 min. The filtrate on cooling gave light brown colored compound 6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-*a*]phenanthrene (**5a**). Yield 2.51 g (90%), m.p. 250 °C (dec.), IR (KBr) 1554, 1596, 1621, 2919, 3053 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.6 (s, 2H, CH_2) and 7.2–8.2 (m, 7H, 6H-Ar and C-10 H), ms spectrum showed *m/z* 279 (M^+), 280 $(M + 1)^+$ and 281 $(M + 2)^+$. Anal. Calcd. C 64.52, H 3.23, N 15.05, S 11.47. Found C 64.32, H 3.00, N 14.80, S 11.27.

*5.7. 11-Bromo-6H-5-oxa-7-thia-8,9,10a-triaza-pentaleno[4,5-a]phenanthrene (**5b**)*

Yield 3.28 g (92%), m.p. 265 °C (dec.), IR (KBr) 1594, 1619, 2763, 2881 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.4 (s, 2H, CH_2) and 7.2–8.0 (m, 6H, 5H-Ar and C-10 H), ms spectrum showed *m/z* 357 (M^+), 358 $(M + 1)^+$, 359 $(M + 2)^+$, 360 $(M + 3)^+$, 361 $(M + 4)^+$. Anal. Calcd. C 50.28, H 2.23, Br 22.35, N 11.73, S: 8.94. Found C 50.00, H 2.03, Br 22.07, N 11.48, S 8.69.

*5.8. 10-Methyl-6H-5-oxa-7-thia-8,9,10a-triaza-pentaleno[4,5-a]phenanthrene (**6a**)*

(12*H*-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**) (2.69 g, 10 mmol) was refluxed with acetic anhydride (25 ml) for 5 h. The reaction mixture was then cooled and neutralized with saturated solution of sodium bicarbonate. The solid obtained was then boiled with 0.5 g of activated animal charcoal and ethanol (50 ml) for 30 min. The filtrate on cooling gave light brown colored 10-methyl-6*H*-5-oxa-7-thia-8,9,10*a*-triaza-pentaleno[4,5-*a*]phenanthrene (**6a**). Yield 2.54 g (87%), m.p. 265 °C (dec.), IR (KBr) 1592, 1617, 2977, 3050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.5 (s, 3H, CH_3), 5.6 (s, 2H, CH_2) and 7.1–8.0 (m, 6H, 6H-Ar), ms spectrum showed *m/z* 293 (M^+), 294 $(M + 1)^+$ and 295 $(M + 2)^+$. Anal. Calcd. C 65.53, H 3.75, N 14.33, S 10.92. Found C 65.38, H 3.50, N 14.03, S 10.72.

*5.9. 1-Bromo-10-methyl-6H-5-oxa-7-thia-8,9,10a-triaza-pentaleno[4,5-a]phenanthrene (**6b**)*

Yield 3.29 (89%), m.p. 292 °C (dec.), IR (KBr) 1595, 1627, 2930, 3070 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.6 (s, 3H, CH_3), 5.5 (s, 2H, CH_2) and 7.1–7.9 (m, 5H, 5H-Ar), MS spectrum showed *m/z* 370 (M^+), 371 $(M + 1)^+$, 372 $(M + 2)^+$, 373 $(M + 3)^+$, 374 $(M + 4)^+$. Anal. Calcd. C 51.61, H 2.69, Br 21.51, N 11.29, S 8.60. Found C 51.36, H 2.42, Br 21.28, N 11.01, S 8.32.

*5.10. 6H-5-oxa-7-thia-8,9,10,10a-tetraaza-pentaleno[4,5-a] phenanthrene (7**a**)*

(12*H*-11-oxa-17-thia-15-aza-cyclopenta[*a*]phenanthrene-16-yl)-hydrazine (**4a**) (2.69 g, 10 mmol) was added to 10% acetic acid solution (30 ml) under stirring at r.t. To this reaction mixture was added sodium nitrate (1.38 g, 20 mmol) in small lots. The reaction was then stirred for further 1 h. The reaction mixture was then neutralized with saturated solution of sodium bicarbonate. The solid obtained was then filtered, dried and then boiled with 0.5 g of activated animal charcoal and ethanol (50 ml) for 30 min. The filtrate on cooling gave light brown colored 6*H*-5-oxa-7-thia-8,9,10,10*a*-tetraaza-pentaleno[4,5-*a*] phenanthrene (**7a**). Yield 2.49 (89%), m.p. 270 °C (dec.), IR (KBr) 1593, 1610, 2977, 3050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.4 (s, 2H, CH_2) and 7.1–7.9 (m, 6H, 6H-Ar), ms spectrum showed *m/z* 280 (M^+), 281 $(M + 1)^+$ and 282 $(M + 2)^+$. Anal. Calcd. C 60.00, H 2.86, N 20.00, S 11.43. Found C 59.80, H 2.62, N 19.72, S 11.19.

*5.11. 1-Bromo-6H-5-oxa-7-thia-8,9,10,10a-tetraaza-pentaleno [4,5-a]phenanthrene (7**b**)*

Yield 3.25 (91%), m.p. 283 °C (dec.), IR (KBr) 1597, 2924, 3067 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.6 (s, 2H, CH_2) and 7.2–8.1 (m, 5H, 5H-Ar), ms spectrum showed *m/z* 358 (M^+), 359 $(M + 1)^+$, 360 $(M + 2)^+$, 361 $(M + 3)^+$ and 362 $(M + 4)^+$. Anal.

Calcd. C 46.80, H 1.95, Br 22.28, N 15.60, S 8.91. Found C 46.54, H 1.68, Br 22.00, N 15.34, S 8.64.

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