Full Paper

Synthesis and Antimicrobial Evaluation of Some New Cyclooctanones and Cyclooctane-Based Heterocycles

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The versatile synthon (*E*)-2-((dimethyl amino)methylene)cyclooctanone (**2**) was used as a key intermediate for the synthesis of cyclooctanones and cyclooctane-based heterocycles with pyrazole, isoxazole, pyrimidine, pyrazolopyrimidine, triazolopyrimidine and imidazopyrimidine derivatives *via* its reactions with several nitrogen nucleophiles. The newly synthesized compounds were screened *in vitro* for their antimicrobial activity against pathogenic microorganisms (*Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*). Most of the tested compounds showed moderate to high antibacterial and antifungal effects against the tested pathogenic microorganisms. Among the synthesized compounds, 2-((*p*-sulfonamidophenyl)methylene)cyclooctanone (**5**) showed excellent activity against *Listeria monocytogenes.*

Keywords: Cyclooctanone / Pyrazole / Pyridine / Pyrimidine / *Listeria monocytogenes* / Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Introduction

Bacteria are the cause of some deadly diseases and widespread epidemics of human civilization. For example, *Listeria monocytogenes* is a facultatively anaerobe, intracellular bacterium that is the causative agent of listeriosis. It is one of the most virulent foodborne pathogens with 20 to 30 percent of clinical infections resulting in death [1]. Annually, listeriosis is the leading cause of death among foodborne bacterial pathogens with fatality rates exceeding even *Salmonella* and *Clostridium botulinum* [2]. Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. MRSA is thought to be one of the resident microbes, which is a harmless to healthy people, but intractable in immunosuppressed patients [3].

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Cyclooctane rings are frequently found in the skeleton of many natural products that have different biological activities [4]. On the other hand, many of nitrogen-containing saturated heterocycles have been found to play fundamental roles in biological process [5].

In view of the above-mentioned facts and in continuation of our interest in the synthesis of a variety of heterocyclic systems for biological and pharmacological evaluations [6–13], we report herein our procedure for the synthesis of cyclooctanones and cyclooctane-based heterocycles with a different substitution pattern starting from (*E*)-2-((dimethyl amino)methylene)cyclooctanone (**2**) and evaluating their selectivity towards pathogenic microorganisms (*Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*).

Results and discussion

Chemistry

The synthetic strategies adopted to obtain the target compounds are outlined in schemes (1–5). The starting compound

(*E*)-2-((dimethylamino)methylene)cyclooctanone (**2**) could be synthesized through treatment of cyclooctanone **1** with dimethylformamide-dimethylacetal (DMF-DMA) under reflux condition (Scheme 1). The structure of compound **2** and their analytical confirmations have been well described [14–16].

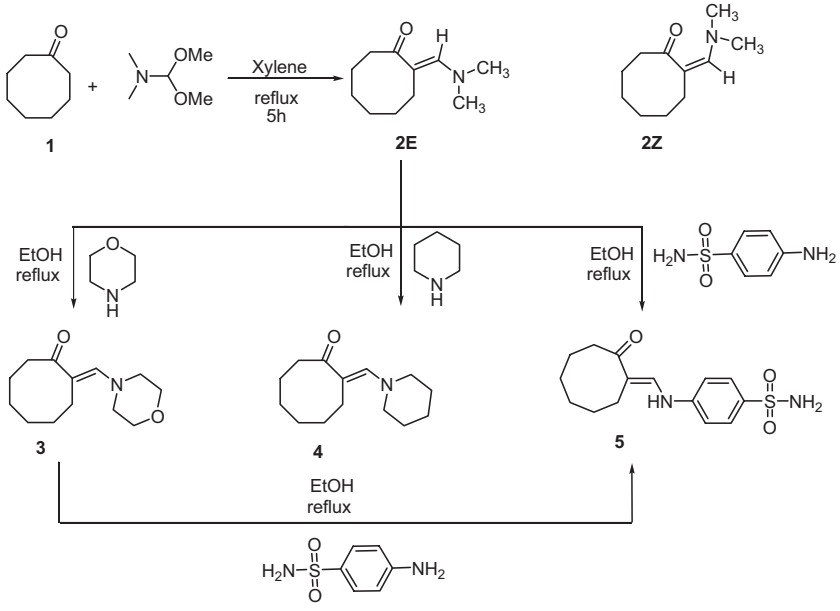
Enaminones are highly reactive building blocks and their utility in organic synthesis has recently earned considerable attention [17–19]. The reactivity of the enaminone **2** towards some nitrogen nucleophiles was investigated. Thus, treatment of enaminone **2** with morpholine and piperidine in refluxing ethanol affords in each case only one isolable product identified as (*E*)-2-(morpholinomethylene)cyclooctanone (**3**) and (*E*)-2-((piperidin-1-yl)methylene)cyclooctanone (**4**), respectively (Scheme 1). The structures of the isolated products were assigned based on their elemental analysis and spectral data. For example, the ^{1}H NMR spectrum of compound **3** displayed signals at δ 2.97–3.98 and 7.45 characteristic for morpholine and C=CH protons, respectively.

When the enaminone **2** or the morpholinyl derivative **3** was treated with *p*-sulfonamide under reflux condition, it afforded 2-((*p*-sulfonamidophenyl)methylene)cyclooctanone (**5**). The ^{1}H NMR spectrum of the latter product exhibited characteristic two doublet signals at δ 7.81 and 11.72 (D_2O-exchangeable) characteristic for C=CH and NH protons, respectively.

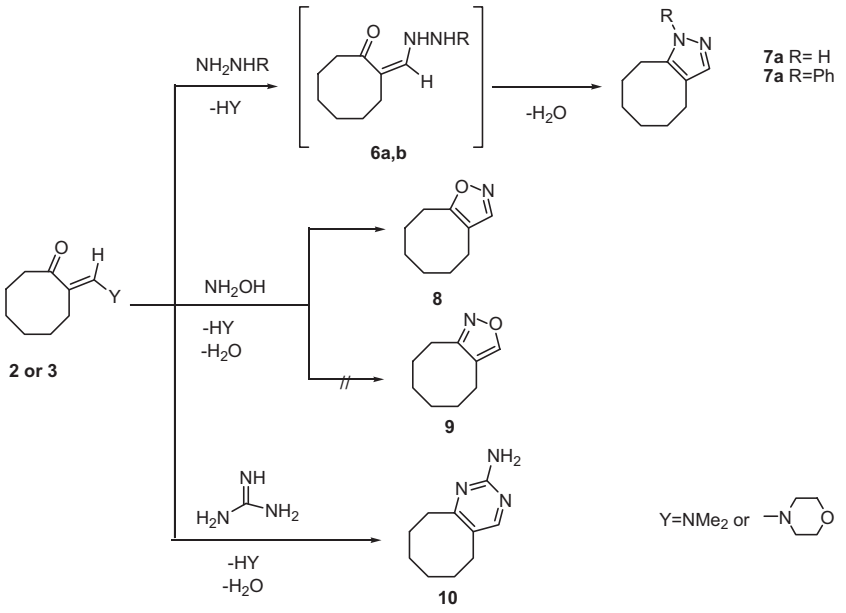
Treatment of the enaminone **2** or the morpholinyl derivative **3** with hydrazine hydrate in refluxing ethanol afforded a single product isolated after chromatography as pale yellow oil identified as 4,5,6,7,8,9-hexahydro-1*H*-cycloocta[*c*]pyrazole (**7a**). Similarly, the enaminone **2** or **3** was reacted with phenyl hydrazine in ethanol at refluxing temperature to afford only one isolable product, identified as 4,5,6,7,8,9-hexahydro-1-phenyl-1*H*-cycloocta[*c*]pyrazole (**7b**) (Scheme 2). The ^{1}H NMR spectrum of compound **7a** showed characteristic singlet signals at 7.39 and 12.10 ppm for H-3 of pyrazole and NH protons, respectively [20–22]. The formation of compounds **7a,b** is assumed to take place *via* a Michael-type addition of the amino group of hydrazines to the enamine double bond in **2** or **3** followed by interamolecular cyclization *via* the loss of dimethylamine or morpholine and water molecules (Scheme 2). In a similar manner, enaminone derivatives **2** or **3** was reacted with hydroxylamine hydrochloride in refluxing ethanol, in the presence of anhydrous sodium acetate, to give only one isolable product identified as 4,5,6,7,8,9-hexahydrocycloocta[*d*]isoxazole (**8**) rather than isomeric form **9** (Scheme 2). The structure of compound **8** was assigned as the correct structure on the basis of the ^{1}H NMR spectrum of the product, where a resonance for H-3 of isoxazole appeared typically at δ 8.41 ppm. The isomeric product was ruled out as H-5 of isoxazole would be expected to resonate at lower filed around δ 9.30 ppm [23, 24].

The enaminone derivatives **2** or **3** reacted also with guanidine in refluxing ethanol, to give a high yield of a single product (as examined by TLC) identified as 5,6,7,8,9,10-hexahydrocycloocta[*d*]pyrimidin-2-amine (**10**) according to its elemental analysis and spectral data. The ^{1}H NMR spectrum of the latter product exhibited characteristic signals at δ 4.88 (D_2O-exchangeable) and at δ 7.96 due to amino and C–H pyrimidine protons, respectively.

The reactivity of enaminone **2** towards some heterocyclic amines was also investigated. Thus, treatment of the enaminone **2** with 5-amino-3-phenyl pyrazole (**11**), 3-amino-1,2,4-triazole (**14**) and 2-amino benzimidazole **17**, in refluxing

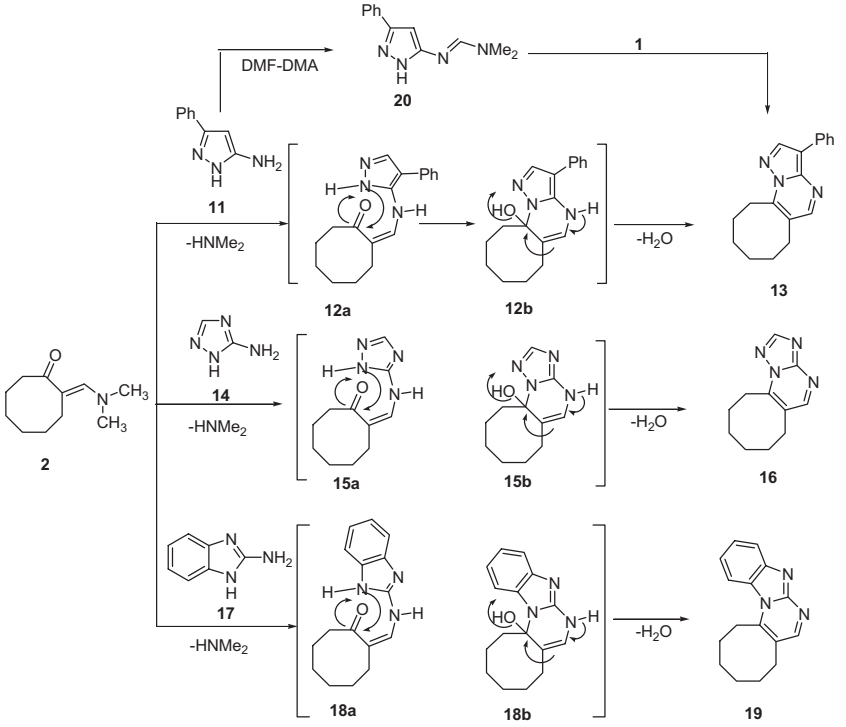


Scheme 1. Synthetic pathway for the formation of compounds **2**, **3**, **4**, and **5**.

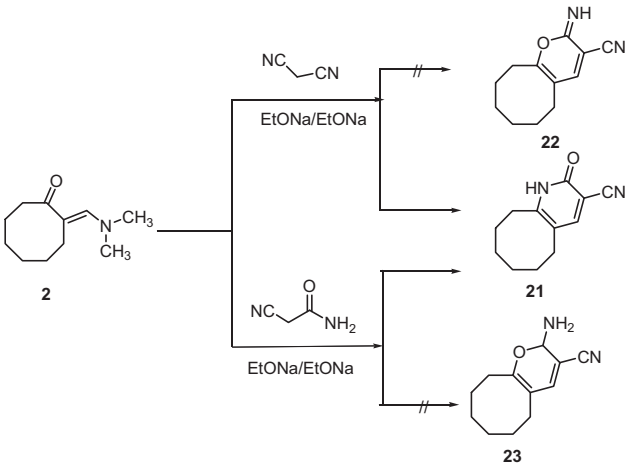


Scheme 2. Synthetic pathway for the formation of compounds **7a,b**, **8** and **10**.

ethanol in the presence of a catalytic amount of piperidine, gave, in each case, a single product identified as 1-phenyl-5,6,7,8,9,10-hexahydrocycloocta[2′,1′-*e*]pyrazolo[1,5-*a*]pyrimidine (**13**), 5,6,7,8,9,10-hexahydrocycloocta[2′,1′-*e*]triazolo[1,5-*a*]pyrimidine (**16**) and 5,6,7,8,9,10-hexahydrocycloocta[2′,1′-*e*]pyrimido[1,2-*a*]benzimidazole (**19**), respectively on the basis of their elemental analysis and spectral data. The reaction of heterocyclic amines **11**, **14** and **17** with enaminone **2** is assumed to take place *via* initial Michael-type addition of amino group to the enamine double bound followed by elimination of the dimethyl amine and water molecules (Scheme 3). Further evidence of the proposed structure **13**



Scheme 3. Synthetic pathway for the formation of compounds **13**, **16** and **19**.



Scheme 4. Synthetic pathway for the formation of compound **21**.

was obtained by an independent synthesis *via* the reaction of *N*-(*N*,*N*-dimethylaminomethylene)imino-3-phenyl-1-*H*-pyrazole (**20**) with cyclooctanone **1** in refluxing acetic acid to afford a product identical in all respects (mp, TLC and spectral data) with that obtained from the reaction of **2** with **11** as shown in Scheme 3.

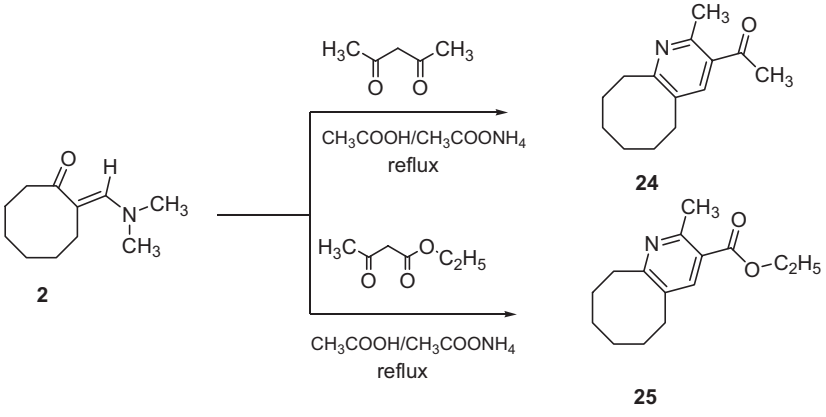
As previously reported, enaminones can be used also as potential precursors for the synthesis of fused pyridine systems when reacting with active methylene compounds [25]. Thus, treatment of enaminone **2** with malononitrile in refluxing sodium ethoxide solution afforded a pale yellow product for which two possible structures **21** and **22** can be formulated (Scheme 4). Structure **22** was ruled out based on the ^{1}H NMR and IR spectral data of the product. The ^{1}H NMR spectrum of the latter product showed the presence of amidic NH at δ 12.39 ppm and the absence of imine signal around δ 3.0 ppm [25]. Also, its IR spectrum revealed amidic carbonyl absorption band at ν 1651 cm^{-1}. Furthermore, the product **21** was further confirmed by an independent synthesis from cyanoacetamide and enaminone **2**. The two reaction products were identical in all respects (mp, TLC and spectra) [see experimental part]. Compound **21** has been reported by another synthetic route from the condensation of cyanoacetamide with sodium salt of 2-formyl-cycloalkanone [26]

Reaction of enaminone **2** with acetyl acetone and ethyl acetoacetate in glacial acetic acid in the presence of ammonium acetate gave cycloocta[*b*]pyridine derivatives **24** and **25**, respectively (Scheme 5). The structures of the isolated products were assigned based on their elemental analysis and spectral data. For example, the ^{1}H NMR spectra of compounds **24** and **25** showed a singlet signals at δ 7.98 and 8.57 ppm respectively, characteristic for H-4 of pyridine. In addition, the mass spectra of compounds **24** and **25** revealed bands at *m*/*z* 217 and 247 respectively, corresponding to their molecular ion peak. On the other hand, compounds **24** and **25** have been previously reported by another synthetic route from 2-aminomethylene-cyclooctanone [27].

Biological testing

Antimicrobial activity

Eight of the newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against pathogenic bacteria such as Gram positive bacteria: *L. monocytogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus*; and Gram negative bacteria: *Ps. aeruginosa*; and anti-fungal activity against *C. albicans*. The minimum inhibitory concentration (MIC) (μg/mL) and inhibition zone diameters values are recorded in Table 1. The results depicted in Table 1 revealed that most of tested compounds of this investigation have moderate to good activity against most of the tested pathogenic bacteria. As compared to the standard drug (ciprofloxacin) with MICs 0.19, 0.097 and 0.39 μg/mL against *L. monocytogenes*, *S. aureus*, *Ps. aeruginosa* respectively,



Scheme 5. Synthetic pathway for the formation of compounds **24** and **25**.

Table 1. Inhibition zone (mm) and minimal inhibitory concentrations (MIC, μg/mL) of some newly synthesized compounds.

Compound no	Zone of inhibition in (mm) and MIC in (μg/mL)				
	Gram+ve Bacteria			Gram−ve Bacteria	Fungi
	MRSA	*L. monocytogenes*	*S. aureus*	*Ps. aeruginosa*	*C. albicans*
5	10 (25)	28 (0.097)	11 (12.5)	13 (3.12)	N
8	12 (12.5)	12 (6.25)	N	13 (3.12)	11 (25)
10	11 (12.5)	14 (1.56)	11 (12.5)	N	11 (50)
13	9 (50)	13 (3.125)	13 (3.125)	11 (3.12)	9 (25)
16	12 (12.5)	13 (3.125)	12 (6.25)	12 (12.5)	10 (25)
19	9 (50)	13 (3.125)	12 (6.25)	12 (6.25)	9 (25)
21	11 (100)	N	N	10 (25)	11 (50)
24	9 (50)	N	N	10 (25)	11 (25)
Vancomycin	18 (12.5)	N	N	N	N
Ciprofloxacin	N	28 (0.19)	28 (0.097)	25 (0.39)	–
Fluconazole	–	–	–	–	19 (1.5)

MIC: Minimal inhibitory concentration value with SEM = 0.02
N, not tested

2-(*p*-sulfonamidophenyl)methylene cyclooctanone (**5**) showed very promising activity with MICs 0.097 and 3.12 μg/mL against *L. monocytogenes* and *Ps. aeruginosa* respectively. Compound **10** shows significant antibacterial activity against *L. monocytogenes* with MIC 1.56 μg/mL. As compared to the standard drug (vancomycin) with MIC 12.5 μg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA), compounds **8**, **10** and **16** showed promising activity with MICs 12.5 μg/mL. The anti-fungal data (Table 1) revealed that some of tested compounds of this investigation have moderate activity against the tested pathogenic fungi (*C. albicans*) as compared to the standard drug fluconazole.

From the antimicrobial activity and structural activity relationship (SAR) of compound **5**, we can conclude that the sulfonamide moiety is essential for the activity against pathogenic bacteria *L. monocytogenes* and *Ps. aeruginosa*. On the other hand the good bacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), exhibited by compounds **8**, **10** and **16** might be due to the presence of isoxazole and pyrimidine moieties. It is interesting to note that an alteration in the molecular configuration of investigated compounds may have a pronounced effect on anti-microbial screening, e.g. compound **16** having fused triazolo[1,5-*a*]pyrimidine moiety showed good antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) than compound **19** with pyrimido[1,2-*a*]benzimidazole moiety.

From structure activity relationship (SAR) point of view, the attachment of phenyl sulfonamide moiety to cyclooctanone ring can be considered as breakthrough in developing new antibiotic agents against *Listeria monocytogenes*.

Experimental section

Chemistry

General

All melting points were measured on a Gallenkamp melting point apparatus (Weiss Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd. Cambridge, England and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform ($CDCl_3$) or dimethyl sulphoxide (DMSO-d_6). Chemical shifts are given in parts per million and were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu) at 70 eV. Elemental analyses were carried out at the Micro-analytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Aluminum Sheets 60 F_{254}, Merck).

Material and reagents

Hydrazine hydrate, phenyl hydrazine, guanidine hydrochloride, acetyl acetone ethylacetoacetate, 2-amiobenzimidazole, 3-aminotriazole were purchased from Aldrich Chemical CO. Triethylamine, morpholine and piperidine, were purchased from British Drug Houses (BDH). Dimethylformamide-dimethylacetal (DMF-DMA) was obtained from Merck CO, Germany. Hydrochloric acid and potassium carbonate were purchased from El-Nasr Pharmaceutical and Chemical CO. (ADWIC). 3-Phenyl-1*H*-pyrazol-5-amine (**11**)

and (*E*)-*N*,*N*-dimethyl-*N*′-(3-phenyl-1*H*-pyrazol-5-yl)formamidine (**20**) were prepared according to literature procedures [28, 29]. Compounds **24** and **25** have been previously reported by another synthetic route from 2-aminomethylene-cyclooctanone [27].

*Preparation of (E)-2-((dimethylamino)methylene)-cyclooctanone (**2**)*

A mixture of cyclooctanone **1** (1.26 g, 10 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (1.33 mL, 10 mmol) was refluxed for 5h, and then allowed to cool. The reaction product was isolated after chromatography to give compound **2** as pale yellow oil. An analytical pure sample was obtained by crystallization [14–16]. Yield (1.61 g, 85%); colorless crystal (hexane), mp: 39–41°C. IR (KBr): *ν* 1680 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.12–2.91 (m, 12H, $6CH_2$), 3.41 (s, 6H, $2CH_3$), 7.52 (s, 1H, CH=C). MS *m/z* (%): 181 [M^+] (75), 145 (100). Anal. Calcd. for $C_{11}H_{19}NO$ (181.27): C, 72.88; H, 10.56; N, 7.73. Found: C, 72.92; H, 10.51; N, 7.80.

*Preparation of compounds **3** and **4***

To a solution of the enaminone **2** (0.90 g, 5 mmol) in ethanol (15 mL) was added morpholine (0.42 g, 5 mmol) or piperidine (0.43 g, 5 mmol). The reaction mixture was refluxed for several hours and the progress of the reaction was monitored by TLC. The solvent was evaporated under reduced pressure and the oil residue was isolated by chromatography (chloroform/methanol, 9:1) to give compound **3** and **4** as pale yellow oil. Analytical pure samples were obtained by crystallization to give colorless crystals of compounds **3** and **4**, respectively.

*(E)-2-(Morpholinomethylene)cyclooctanone (**3**)*

Yield (0.80 g, 72%); colorless crystal (hexane); mp: 42–44°C. IR (KBr): *ν* 1667 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15–2.93 (m, 12H, $6CH_2$), 2.97 (t, 4H, $2CH_2$ morpholine), 3.98 (t, 4H, $2CH_2$, morpholine), 7.45 (s, 1H, CH=C). MS *m/z* (%): 223 [M^+] (75), 151 (51), 126 (100), 113 (43), 59 (70). Anal. Calcd for $C_{13}H_{21}NO_2$ (223.31): C, 69.92; H, 9.48; N, 6.27. Found: C, 69.94; H, 9.44; N, 6.33.

*(E)-2-((Piperidin-1-yl)methylene)cyclooctanone (**4**)*

Yield (0.90 g, 82%); colorless crystal (hexane); mp: 45–47°C. IR (KBr): *ν* 1664 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.14–3.93 (m, 22H, $11CH_2$), 7.45 (s, 1H, CH=C). MS *m/z* (%): 221 [M^+] (25), 178 (13), 151 (21), 125 (35), 86 (83), 60 (100). Anal. Calcd. for $C_{14}H_{23}NO$ (221.34): C, 75.97; H, 10.47; N, 6.33. Found: C, 75.91; H, 10.44; N, 6.37.

*2-((p-Sulfonamidophenyl)methylene)cyclooctanone (**5**)*

To a solution of the enaminone derivative **2** or **3** (5 mmol) in ethanol (10 mL) was added *p*-sulfonamide (0.86 g, 5 mmol). The reaction mixture was refluxed for 8 h, then the solvent was distilled off under reduced pressure and the residual brown solid was taken in methanol. The solid product so formed was filtered off, washed thoroughly with methanol, dried and finally recrystallized from ethanol to afford compound **5**.

Yield (1.15 g, 75%); pale yellow crystals (ethanol); mp: 179–180°C. IR (KBr): *ν* 3360, 3161 (NH, NH_2), 1644 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.20–3.11 (m, 12H, $6CH_2$), 7.15–7.55 (m, 4H, ArH's, 2H, NH_2-SO_2, D_2O-exchangeable), 7.81 (d, 1H, CH-enamine), 11.72 (d, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 308 [M^+] (59), 266 (30), 211 (98), 130 (100), 91 (46). Anal. Calcd for $C_{15}H_{20}N_2O_3S$ (308.40): C, 58.42; H, 6.54; N, 9.08. Found: C, 58.52; H, 6.57; N, 9.10.

*Reaction of compounds **2** and **3** with hydrazines*

To a solution of the enaminone derivative **2** or **3** (5 mmol) in absolute ethanol (20 mL) was added (5 mmol) of the appropriate hydrazines. The reaction mixture was refluxed for several hours. The solvent was distilled off at reduced pressure and the residual viscous liquid was taken in methanol and isolated by chromatography (chloroform) to afford the corresponding pyrazole derivatives **7a**,**b**. The synthesized compounds together with their physical and spectral data are listed below:

*4,5,6,7,8,9-Hexahydro-1H-cycloocta[c]pyrazole (**7a**)*

Yield (0.51 g, 68%); colorless crystal (hexane); mp: 49–51°C. IR (KBr): *ν* 3320 (NH) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.30–3.01 (m, 12H, $6CH_2$), 7.39 (s, 1H, CH pyrazole), 12.10 (s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 150 [M^+] (100). Anal. Calcd. for $C_9H_{14}N_2$ (150.22): C, 71.96; H, 9.39; N, 18.65. Found: C, 71.90; H, 9.42; N, 18.71.

*4,5,6,7,8,9-Hexahydro-1-phenyl-1H-cycloocta[c]-pyrazole (**7b**)*

Yield (0.81 g, 72%); pale yellow crystal (hexane); mp: 55–57°C. ^1H NMR (DMSO-d_6): δ 1.39–3.61 (m, 12H, $6CH_2$), 7.45 (s, 1H, CH-pyrazole), 6.98–7.30 (m, 5H, ArH's). MS *m/z* (%): 226 [M^+] (24), 183 (21), 170 (17), 77 (100). Anal. Calcd for $C_{15}H_{18}N_2$ (226.32): C, 79.61; H, 8.02; N, 12.38. Found: C, 79.57; H, 7.97; N, 12.35.

Reaction of (E)-2-dimethylaminomethylene cyclooctanone with hydroxylamine hydrochloride and guanidine

General procedure

To a mixture of the enaminone **2** or **3** (5 mmol) and hydroxylamine hydrochloride or guanidine hydrochloride (5 mmol) in ethanol (10 mL) was added anhydrous potassium carbonate (5 mmol). The resulting mixture was refluxed for 5 h and allowed to cool to room temperature then diluted with water (30 mL). The solid products that formed were collected by

filtration, washed with water, dried and recrystallized from the proper solvent to afford compounds **8** and **10**, respectively.

*4,5,6,7,8,9-Hexahydrocycloocta[d]isoxazole (**8**)*

Yield (0.55 g, 73%); white solid (ethanol/dioxane); mp: 250–252°C. ^{1}H NMR (DMSO-d_6): δ 1.37–3.52 (m, 12H, 6CH_2), 8.41 (s, 1H, CH-isoxazole). MS *m/z* (%): 151 [M^+] (35), 69 (100). Anal. Calcd. for $C_9H_{13}NO$ (151.21): C, 71.49; H, 8.67; N, 9.26. Found: C, 71.43; H, 8.70; N, 9.23.

*5,6,7,8,9,10-Hexahydrocycloocta[d]pyrimidin-2-amine (**10**)*

Yield (0.84 g, 95%); pale yellow crystals (ethanol); mp: 173–174°C. IR (KBr): *ν* 3336, 3160 (NH_2) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.24–2.73 (m, 12H, 6CH_2), 4.88 (s, 2H, NH_2, D_2O-exchangeable), 7.96 (s, 1H, CH-pyrimidine). ^{13}C NMR ($CDCl_3$): δ 25.73, 25.99, 28.34, 29.85, 32.29, 33.90 (6 CH_2), 123.66, 157.57, 162.03, 170.52 (pyrimidine carbons). MS *m/z* (%): 177 [M^+] (73), 149 (89), 95 (100). Anal. Calcd. for $C_{10}H_{15}N_3$ (177.25): C, 67.76; H, 8.53; N, 23.71. Found: C, 67.71; H, 8.59; N, 23.67.

*Reaction of (E)-2-dimethylaminomethylene cyclooctanone with heterocyclic amines: Preparation of compounds **13**, **16** and **19**.*

Method A

General procedure: To a mixture of the enaminone **2** (0.90 g, 5 mmol) and the appropriate heterocyclic amines **11**, **14** and **17** (5 mmol) in ethanol (20 mL), a catalytic amount of piperidine was added. The reaction mixture was refluxed for several hours (the reactions were followed by TLC). The solvent was distilled off at reduced pressure and the residual viscous liquid was taken in methanol and the resulting solid was collected by filtration, washed thoroughly with ethanol, dried and finally crystallized from ethanol to afford compounds **13**, **16** and **19**, respectively. The synthesized compounds **13**, **16** and **19** together with their physical and spectral data are listed below.

Method B

A mixture of cyclooctanone **1** (10 mmol) and an equivalent molar ratio of 5-*N*-(*N*,*N*-dimethylaminomethylene)amino-3-methyl-1*H*-pyrazole (**20**) in acetic acid (20 mL) was heated under reflux for 6 h. The solvent was removed by distillation under reduced pressure and the remainder was left to cool. The precipitated solid product was collected by filtration. Crystallization from ethanol afforded product identical in all respects (mp, mixed mp, TLC, IR, and mass spectra with **13**).

*1-Phenyl-5,6,7,8,9,10-hexahydrocycloocta[2′,1′-e]-pyrazolo[1,5-a]pyrimidine (**13**)*

Yield (1.03 g, 75%); pale yellow crystals (ethanol); mp: 125–126°C. ^{1}H NMR (DMSO-d_6): δ 1.22–3.78 (m, 12H, 6CH_2), 6.91–7.43 (m, 5H, ArH′s), 8.00 (s, 1H, pyrazole-H), 8.37 (s, 1H, pyrimidine-H). MS *m/z* (%): 277 [M^+] (100), 149 (83). Anal. Calcd. for $C_{18}H_{19}N_3$ (277.36): C, 77.95; H, 6.90; N, 15.15. Found: C, 77.85; H, 6.94; N, 15.11.

*5,6,7,8,9,10-Hexahydrocycloocta[2′,1′-e]triazolo[1,5-a]-pyrimidine (**16**)*

Yield (0.80 g, 79%); pale yellow crystals (ethanol); mp: 121–122°C. ^{1}H NMR ($CDCl_3$): δ 1.31–3.28 (m, 12H, 6CH_2), 8.57 (s, 1H, pyrimidine-H), 8.68 (s, 1H, triazole-H). ^{13}C NMR ($CDCl_3$): δ 25.77, 25.88, 26.24, 26.38, 27.14, 31.86 (6 CH_2), 122.93, 149.22, 154.65, 155.45, 155.62 (triazole and pyrimidine carbons). MS *m/z* (%): 202 [M^+] (100), 174 (89), 159 (62), 146 (43). Anal. Calcd. for $C_{11}H_{14}N_4$ (202.26): C, 65.32; H, 6.98; N, 27.70. Found: C, 65.26; H, 6.88; N, 27.63.

*5,6,7,8,9,10-Hexahydrocycloocta[2′,1′-e]pyrimido [1,2-a]-benzimidazole (**19**)*

Yield (0.90 g, 72%); yellow crystals (ethanol); mp: 133–134°C. ^{1}H NMR ($CDCl_3$): δ 1.25–3.35 (m, 12H, 6CH_2), 7.25–7.55 (m, 4H, ArH′s), 8.48 (s, 1H, pyrimidine-H). ^{13}C NMR ($CDCl_3$): 25.83, 25.92, 29.32, 29.78, 32.96, 35.30 (6 CH_2), 110.44, 120.32, 121.19, 125.88, 126.80, 130.02, 144.49, 150.94, 170.91 (aromatic, imidazole and pyrimidine carbons). MS *m/z* (%): 251 [M^+] (100), 222 (25), 77 (15). Anal. Calcd. for $C_{16}H_{17}N_3$ (251.33): C, 76.46; H, 6.82; N, 16.72. Found: C, 76.39; H, 6.78; N, 16.84.

*1,2,5,6,7,8,9,10-Octahydro-2-oxocycloocta[b]-pyridine-3-carbonitrile (**21**)*

To a solution of **2** or **3** (5 mmol) in ethanolic sodium ethoxide [prepared from sodium metal (0.11g) and absolute ethanol (20 mL)] was added malononitrile or cyanoacetamide (5 mmol). The reaction mixture was refluxed for 5 h, and then poured into ice-cold water. The solution was acidified with diluted HCl. The formed solid was collected by filtration, washed with water, dried and finally recrystallized from ethanol/dioxane, to afford the same product **21** identical in all respects (TLC, mp and spectral data), yield (75%, 71%, respectively), Brown crystals (ethanol/dioxane); mp: 252–254°C. IR (KBr): *ν* 3410 (NH), 2219 (CN), 1651 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.11–3.28 (m, 12H, 6CH_2), 7.98 (s, 1H, pyridine-H), 12.39 (s, 1H, D_2O-exchangeable, NH). MS *m/z* (%): 202 [M^+] (100), 187 (17), 173 (39), 159 (63), 146 (21). Anal. Calcd. for $C_{12}H_{14}N_2O$ (202.25): C, 71.26; H, 6.98, 13.85. Found: C, 71.35; H, 7.12; .N, 13.73.

Reaction of (E)-2-dimethylaminomethylene cyclooctanone (2) with acetyl acetone and ethyl acetoacetate

General procedure: To a mixture of **2** or **3** (5 mmol) and ammonium acetate (0.5 g) in glacial acetic acid (20 mL), was added acetyl acetone or ethyl acetoacetate (5 mmol).

The reaction mixture was refluxed for several hours and followed by TLC. The solvent was evaporated under reduced pressure, the oil residue was isolated with chromatography on silica gel using chloroform and the product was isolated as pale yellow oil. An analytical pure sample was obtained by crystallization to give the corresponding pyridine derivative **24** and **25**. The synthesized compounds **24** and **25** together with their physical and spectral data are listed below.

*1-(5,6,7,8,9,10-Hexahydro-2-methylcycloocta[b]-pyridin-3-yl)ethanone (**24**)*

Yield (0.83 g, 77%); colorless crystal (hexane); mp: 55–57°C. IR (KBr): ν 1685 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.19–3.37 (m, 12H, $6CH_2$), 2.66, 2.69 (s, 6H, $2CH_3$), 7.98 (s, 1H, CH-pyridine). ^{13}C NMR ($CDCl_3$): δ 20.26 (CH_3), 25.76, 25.81, 29.32, 30.38, 34.40, 32.01, 34.47, (6 CH_2 and acetyl CH_3), 130.61, 133.27, 137.68, 155.32, 163.49 (pyridine carbons), 200.42 (acetyl C=O). MS *m/z* (%): 217 [M^+] (25), 202 (31), 81 (100), 67 (93). Anal. Calcd. for $C_{14}H_{19}NO$ (217.31): C, 77.38; H, 8.81; N, 6.45. Found: C, 77.43; H, 8.73; N, 6.47.

*Ethyl-5,6,7,8,9,10-hexahydro-2-methylcycloocta[b]pyridine-3-carboxylate (**25**)*

Yield (0.90 g, 73%); colorless crystal (hexane); mp: 61–63°C. IR (KBr): ν 1721 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15–3.36 (m, 12H, $6CH_2$), 1.23 (t, $J = 8$ Hz, 3H, CH_3), 2.93 (s, 3H, CH_3), 4.32 (q, $J = 8$ Hz, 2H, CH_2), 8.57 (s, 1H, CH-pyridine). ^{13}C NMR ($CDCl_3$): δ 14.26 (CH_3CH_2), 24.19, 25.74, 25.78, 30.39, 31.19, 34.44, 40.82 (6 CH_2 and CH_3), 60.91 (CH_3CH_2), 123.21, 133.21, 138.75, 156.65, 163.83 (pyridine carbons), 166.86 (ester C=O). MS *m/z* (%): 247 [M^+] (100), 216 (73), 174 (23), 81 (71), 67 (92). Anal. Calcd. for $C_{15}H_{21}NO_2$ (247.33): C, 72.84; H, 8.56; N, 5.66. Found: C, 72.73; H, 8.62; N, 5.72.

Biological screening

Material and reagents

Tested strains include: bacterial strains as Gram positive bacteria; Methicillin resistant *S. aureus* (MRSA) from fecal sample of dead mare due to toxic shock syndrome containing *mec-A* gene isolated and completely identified by biochemical identification including coagulase test, antibiotic resistance test as well as molecular analysis. *L. monocytogenes* and *S. aureus* isolated from mastitic cow milk and were identified using biochemical analysis. Gram negative bacterium; *Ps. aeruginosa* was isolated from mastitic cow milk and completely identified by biochemical analysis using indole test, methyl red, Voges-Proskauer, Simon citrate test and production of hydrogen sulphide on triple sugar iron agar media. Fungal strain; *C. albicans* isolated from vaginal swab of dead mare due to MRSA. Tested strains isolated and identified at the department of microbiology and immunology, veterinary division, National Research Centre (NRC), Dokki, Egypt.

Ciprofloxacin, vancomycin and fluconazole were used as reference drugs (Oxoid).

Antimicrobial activity

Antibacterial as well as antifungal activity of eight tested compounds in DMSO (100 μg/mL) was *in vitro* evaluated using agar well diffusion method [30, 31]. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial or fungal growth around the disks in mm. Vancomycin against MRSA and ciprofloxacin (100 μg/mL) were used as positive control standard antibacterial, fluconazole (100 μg/mL) was used as standard antifungal.

Using microtiter dilution plate method [32], the minimum inhibitory concentration (MIC) considered as quantitative method was used for evaluation of the antibacterial activity of the synthesized compounds against inhibited organisms. MIC gives more details about the quantitative hindrance ability of the tested compounds against the tested bacterial strains: MRSA, *Ps. aeruginosa, L. monocytogenes* and *S. aureus*. The results of the agar well diffusion test and MIC of tested compounds to reveal their antibacterial and antifungal activities are shown in Table 1.

Methods

Preparation of bacterial suspensions

Suspension of the above mentioned microorganisms was prepared by inoculating fresh stock cultures into separate broth tubes, each containing (7 mL) of Muller Hinton broth for bacterial strains and Sabouraud dextrose broth for fungal strain. The inoculated tubes were incubated at 37°C and 28°C for 24 h for bacterial and fungal strains respectively.

Muller Hinton agar and Sabouraud dextrose agar (Oxiod) were melted and poured each in empty sterile Petri dishes (15 mm) and left for 24 h. Wells of diameter 6 mm were formed in wells using sterile Pasteur pipette. A specific culture of each organism was spread with a dry sterile swab on the surface of the previously prepared plates. 50 μL of solutions of the tested compound were then placed onto the wells. Also, antimicrobial standard were put in the centre of the plate agar and incubated at 37–28°C for 24 h for bacterial and fungal strains, respectively. After incubation, the plates were examined visually and the zone of inhibition was measured. The test was repeated three times for each compound.

Determination of the minimum inhibitory concentration (MIC)

Determination of MIC of compounds against tested strains was achieved using 96-well sterile micro plates. The plates were incubated at 37–28°C for 24 h for bacterial and fungal

strains respectively. After incubation, the plates were examined visually for bacterial or fungal growth precipitation and the experiment was repeated three times.

It was carried out for the tested compounds with initial concentration 100 μg/mL, two fold serial dilutions of the tested compounds, reference antibiotic (vancomycin and ciprofloxacin) and fluconazole were prepared using Muller Hinton broth. The tubes were then incubated with the tested organisms, grown in their suitable broth at 37°C for 24 h for bacteria and at 28°C for 24 h for fungi (about 1×10^5 cells/mL), each well receiving 100 μL of the above inoculums and incubated at 37–28°C for 24–48 h for bacterial and mycotic growth, respectively. The lowest concentration that showed no growth was taken as MIC.

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