Ahmed H. Halawa, Shimaa Mohamed Abd El-Gilil, Ahmed H. Bedair, Mohamed Shaaban, Marcel Frese, Norbert Sewald, Essam M. Eliwa and Ahmed M. El-Agrody*

# Synthesis, biological activity and molecular modeling study of new Schiff bases incorporated with indole moiety

DOI 10.1515/znc-2017-0025
Received February 17, 2017; revised April 13, 2017; accepted April 25, 2017

**Abstract:** A new series of heterocyclic Schiff bases **2–9** containing indole moiety were synthesized by facile and efficient condensation of indole-3/2/5-carboxaldehyde (**1a**/**1b**/**1c**) with different aromatic and heterocyclic primary amines using conventional and/or microwave irradiation methods. The structures of the obtained compounds were assigned by sophisticated spectroscopic and spectrometric techniques (1D-NMR, 2D-NMR and MS). The synthesized compounds were screened for their cytotoxicity and antibacterial activities. In vitro cytotoxicity screening revealed that compound **5** exhibited moderate activity against KB-3-1 cell line ($IC_{50}$ = 57.7 μM) while 5-indolylimino derivative **7** indicated close to the activity ($IC_{50}$ = 19.6 μM) in comparison with the positive control (+)-Griseofulvin ($IC_{50}$ = 19.2 μM), while the tested compounds **5**, **6b**, **7** and **9** revealed good or moderate antibacterial activity. In addition, molecular docking study of Schiff bases **2–9** was performed by Molecular Operating Environment (MOE 2014.09) program on the matrix metalloproteinase-8 (MMP-8) (Protein Data Bank (PDB) ID: 1MNC) in an attempt to explore their mode of action as anticancer drugs.

**Keywords:** antibacterial activity; cytotoxicity activity; microwave irradiation; molecular docking; Schiff base; thermal synthesis.

***Corresponding author: Ahmed M. El-Agrody,** Chemistry Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt, E-mail: elagrody_am@yahoo.com
**Ahmed H. Halawa and Ahmed H. Bedair:** Chemistry Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt
**Shimaa Mohamed Abd El-Gilil:** Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, 11754, Cairo, Egypt
**Mohamed Shaaban:** Organic and Bioorganic Chemistry, Faculty of Chemistry, Bielefeld University, D-33501 Bielefeld, Germany; and Chemistry of Natural Compounds Department, Division of Pharmaceutical Industries, National Research Centre, El-Behoos St. 33, Dokki-Cairo 12622, Egypt
**Marcel Frese and Norbert Sewald:** Organic and Bioorganic Chemistry, Faculty of Chemistry, Bielefeld University, D-33501 Bielefeld, Germany
**Essam M. Eliwa:** Chemistry Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt; and Organic and Bioorganic Chemistry, Faculty of Chemistry, Bielefeld University, D-33501 Bielefeld, Germany

## 1 Introduction

Indole derivatives are found in many natural products such as fungal metabolites, indole alkaloids and marine organisms [1–6]. The indole nucleus has been reported to have an important role in medicinal chemistry involving antimicrobial [7, 8], anti-inflammatory [9, 10], antioxidant [11–13], anticancer [14–17], antibiotic [2] and anti-HIV [18, 19] activities. Besides, C-3-substituted indoles are versatile intermediates for the synthesis of many pharmacologically active compounds [20–24]. Schiff bases are a vital class of organic molecules that have azomethine group and versatile applications such as polymer stabilizers, catalysts and intermediates in organic synthesis, ligands in coordination chemistry, and pigments or dyes [25–27]. Moreover, Schiff bases have attracted considerable attention due to their extensive biological activities, including antibacterial, antifungal, antimalarial, antiviral and antipyretic properties [28–30].

The aim of the present investigation was to design new Schiff bases incorporated with indole moiety and check their biological activity in a trial to obtained new derivatives with high activity and less toxicity. Additionally, we investigated the molecular docking of the synthesized compounds using an MOE module (MOE2014.09) to understand the mode of action of Schiff bases through their various interactions with active sites of MMP-8 (PDB ID: 1MNC).

# 2 Results and discussion

## 2.1 Chemistry

In thermal or microwave-assisted synthesis, 1*H*-indole-3-carboxaldehyde (**1a**) and primary amines, namely, 2-amino-6-ethoxy/methoxybenzothiazole, 4-aminoacetanilide, 4,4′-diamino-3,3′-dimethoxybiphenyldihydrochloride, benzophenone hydrazone, 4-aminobenzoic acid and methyl 4-aminobenzoate, were refluxed and/or irradiated in the presence of a catalytic amount of acetic acid or triethylamine to afford the corresponding Schiff bases **2–6** as depicted in Scheme 1.

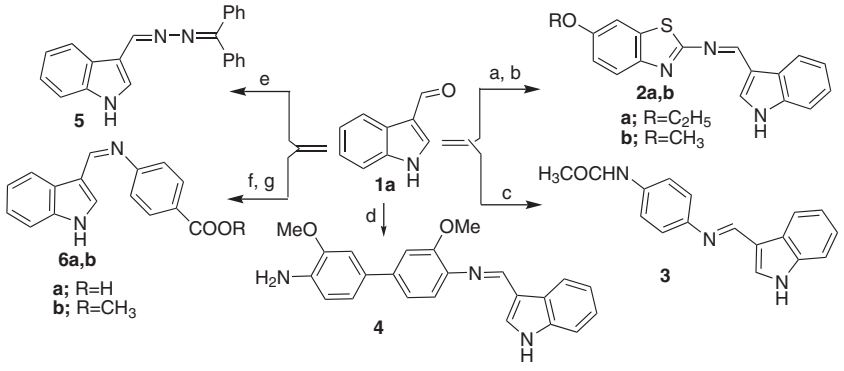
In a similar manner, the treatment of indole-carboxaldehydes (**1a–c**), namely, 1*H*-indole-3-carboxaldehyde (**1a**), 1*H*-indole-2-carboxaldehyde (**1b**) and 1*H*-indole-5-carboxaldehyde (**1c**), with 5-aminoindole affords the corresponding Schiff bases **7–9** as shown in Scheme 2.

The observed physical properties of the title compounds and comparison between the thermal and microwave methods are given in Tables S1 and S2 (see Supplementary information).

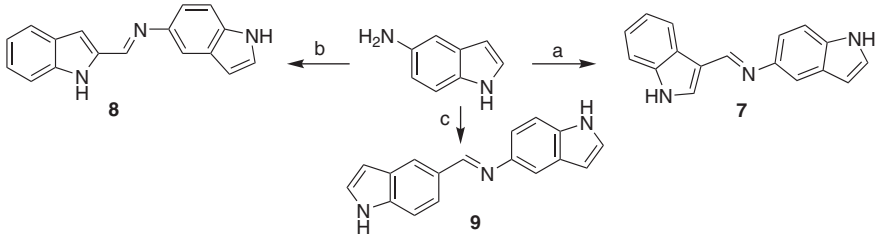
The results in Table S2 indicated that short reaction times, high purity of products, high yields, less side products, simplified synthetic procedure, economical with environmental technology, are all advantages offered by microwave irradiation method, which is better than thermal method.

The proton and carbon-13 nuclear magnetic resonance ($^1$H and $^{13}$C NMR) spectra of **2–6** revealed the presence of a broad 1H singlet at $\delta$ 12.28–9.76 ppm due to the NH of indole moiety and a singlet at $\delta$ 9.94–8.72 ppm due to the proton of azomethine groups and the respective carbons at $\delta$ 160.8–154.6 ppm.

According to the heteronuclear multiple-quantum correlation spectroscopy (HMQC) experiment of **2a**, the proton resonance at $\delta$ 9.19 (H-8) ppm is directly connected to carbon at $\delta$ 160.8 (C-8) ppm and did not have any correlation with other protons according to the correlation spectroscopy ($^1$H,$^1$H-COSY) spectrum (Supplementary information, Table S3, Figures S3 and S4). The formation of Schiff base **2a** was further confirmed by HMBC correlation, as shown in the Supplementary information, Figures S5 and S6, where methine proton was coupled with C-3 ($\delta$ 114.8 ppm) in indole ring through two-bond




**Scheme 1:** Schematic representation for the synthesis of Schiff bases **2–6**. *Reagents*: (a) reflux or MW, MeOH, AcOH, 2-amino-6-ethoxybenzothiazole; (b) reflux or MW, MeOH, AcOH, 2-amino-6-methoxybenzothiazole; (c) reflux or MW, AcOH, 4-aminoacetanilide; (d) reflux or MW, MeOH, TEA, 4,4′-diamino-3,3′-dimethoxybiphenyldihydrochloride; (e) reflux or MW, MeOH, AcOH, benzophenone hydrazone; (f) reflux or MW, MeOH, AcOH,4-aminobenzoic acid; (g) reflux or MW, MeOH, AcOH and methyl-4-aminobenzoate.




**Scheme 2:** Schematic representation for the synthesis of Schiff bases **7–9**. *Reagents*: (a) reflux or MW, MeOH, AcOH, 1*H*-indole-3-carboxaldehyde (**1a**); (b) reflux or MW, MeOH, AcOH, 1*H*-indole-2-carboxaldehyde (**1b**); (c) reflux or MW, MeOH, AcOH, 1*H*-indole-5-carboxaldehyde (**1c**).

correlation ($^2J$) and with carbons C-2/3a/2′ through three-bond correlation ($^3J$), proving the formation of imine functional group between the reactants. In addition, the electrospray ionization mass spectrometry (ESI-MS) spectrum of compound **2a** shows the molecular ion peaks at *m/z*: 322 $[M+H]^+$ in the positive mode and 320 $[M-H]^-$ in the negative mode, respectively confirming the molecular weight of **2a** as 321 Dalton. Its high resolution electron ionization mass spectrometry (HR-EIMS) was compatible with the molecular formula $C_{18}H_{15}N_3OS$ ($M^+$: 321.09200).

The formation of Schiff base **5** was confirmed by two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) spectra (Supplementary information, Figures S25–S28), where the imine proton at $\delta$ 8.84 ppm was directly connected to the $sp^2$ carbon at $\delta$ 156.6 ppm according to the HMQC experiment. The mass spectrum of **5** provided additional evidence to confirm the structure, where the ESI-MS spectrum displays the molecular ion peak at *m/z*: 324 $[M+H]^+$. HR-EI-MS spectrum of **5** further established the structure, where it showed a molecular ion peak at *m/z*: 323.14164, which corresponds to the molecular formula $C_{22}H_{17}N_3$.

Besides, one-dimensional nuclear magnetic resonance spectroscopy (1D NMR) spectra of compounds **7–9** exhibit two broad signals at $\delta$ 11.70–11.39 and 11.16–11.08 ppm due to 2 NH of the indole moieties, also a singlet at $\delta$ 8.82–8.69 ppm due to the proton of imine groups and the respective carbons at $\delta$ 157.0–149.1 ppm.

The structure of compound **8** was further confirmed by 2D-NMR spectra (Supplementary information, Table S4, Figurs S45–S48), where in the $^1$H,$^1$H-COSY experiment, methine proton at $\delta$ 8.69 ppm did not have any correlation with other protons. In the HMQC spectrum, we saw that the proton with a chemical shift of $\delta$ 8.69 ppm was attached directly to the $sp^2$ carbon with resonance at $\delta$ 149.1 ppm. According to the heteronuclear multiple bond correlation spectroscopy (HMBC) experiment, the imine proton was coupled with three different carbons C-2/3/5′, proving the formation of Schiff base **8**. MALDI-MS spectra exhibited the molecular ion peak at *m/z*: 260 $[M+H]^+$ for **7** and **8**, while the HR-EI-MS spectrum of **9** further confirmed the structure, where it showed the molecular ion peak at *m/z*: 259.10927, which corresponds to the molecular formula $C_{17}H_{13}N_3$.

## 2.2 Biological activities

### 2.2.1 Cytotoxic activity

Based on the reported cytotoxic and biological activities of the indole nucleus and Schiff base compounds [1–24, 28–30], new Schiff bases with indole moieties **2–9** were synthesized and selected to carry out a preliminary screening for their cytotoxic effect against human cervix carcinoma cell line KB-3-1 using the Resazurin assay [31, 32]. In vitro cytotoxicity activity of the synthesized Schiff bases **2–9** was evaluated against KB-3-1 cell line, and (+) Griseofulvin was used as reference cytotoxic compound. The $IC_{50}$ values for all tested compounds are shown in Table 1. The results indicate that 5-indolylimino derivative **7** exhibited excellent activity ($IC_{50}$ = 19.6 μM) and was very close to the reference [(+) Griseofulvin $IC_{50}$ = 19.2 μM], so it is the most potent one, while hydrazineylidene derivative **5** showed moderate activity ($IC_{50}$ = 57.7 μM). In contrast, the other compounds showed no activity against KB-3-1 cell line.

**Table 1:** In vitro cytotoxicity of Schiff bases **2–9** against KB-3-1 cell line.

| Compound | $IC_{50}$ (μM) KB-3-1 | Compound | $IC_{50}$ (μM) KB-3-1 |
|---|---|---|---|
| **2a** | – | **6b** | >100 |
| **2b** | – | **7** | 19.6 |
| **3** | – | **8** | – |
| **4** | – | **9** | – |
| **5** | 57.7 | (+)-Griseofulvin | 19.2 |
| **6a** | – | DMSO | – |

### 2.2.2 Antibacterial activity

Schiff bases **2–9** were screened to antibacterial assay by agar diffusion method [33] employing Gram-positive bacteria, namely, *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus warneri* as well as Gram-negative bacteria, namely, *Escherichia coli* and *Pseudomonas agarici*. Activities were

**Table 2:** Inhibition zones of compounds **2–9** against bacterial strains (∅: mm).

| Compound | *E. coli* | *P. agarici* | *B. subtilis* | *M. luteus* | *S. warneri* |
|---|---|---|---|---|---|
| **2a** | – | – | – | – | – |
| **2b** | – | 7 | – | – | – |
| **3** | 6.5 | 14 | – | – | – |
| **4** | – | 14 | – | – | – |
| **5** | – | 7 | 7 | 7 | 8 |
| **6a** | – | 10 | 7 | – | – |
| **6b** | 6.5 | 14 | 6.5 | – | 7 |
| **7** | – | 14 | 7 | 10 | 7 |
| **8** | – | – | – | – | 7 |
| **9** | – | 17 | – | 14 | 15 |
| Gentamycin | 18 | 23 | 20 | 18 | 16 |
| DMSO | – | – | – | – | – |

compared to gentamycin as positive control, while DMSO was used as negative control and tabulated in Table 2.

Interestingly, compounds **5**, **6b** and **7** showed moderate activity against all the bacterial strain except *Escherichia coli* with **5**, **7** and *Micrococcus luteus* with **6b**, which were not active. Compound **4** exhibited good activity against *Pseudomonas agarici* and did not show any activity against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus warneri*, while compound **9** showed activity against *Pseudomonas agarici*, *Micrococcus luteus* and *Staphylococcus warneri* close to the gentamycin and not active against *Escherichia coli* and *Bacillus subtilis*. In addition, the other compounds showed no activity mostly with all bacterial strains.

## 2.3 Molecular docking study

Docking study of Schiff bases **2–9** was performed by MOE 2014.09 program [34] on the matrix MMP-8 (PDB ID: 1MNC) [35, 36] in a trial to explore their mode of action as anticancer drugs. Based on the resulting data (see supplementary information, Table S5 and the score energy in Figure S57), compounds **3**, **9**, **6b**, **5** and **2b** showed maximum affinity with the active sites of the protein MMP-8, and the docking score energy for the tested compounds followed this order: **3** > **9** > **6b** > **5** > **2b** > **2a** > **8** > **7** > **6a** > **4** > (+)-Griseofulvin.

### 2.3.1 Docking of (+)-Griseofulvin into MMP-8 active site

Docking of (+)-Griseofulvin into MMP-8 active site showed the presence of several hydrophobic interactions involving methyl groups, oxygen atoms of carbonyl functions, chlorine atom and other atoms of the compound with the following amino acid residues: Gly 179, Ile 180, Leu 181, Ala 182, Glu 219, Pro 238, Asn 239 and Tyr 240 (see supplementary information Figure S58).

### 2.3.2 Docking of compound 2a into MMP-8 active site

Docking of compound **2a** into MMP-8 active site revealed the interaction of sulfur atom as a hydrogen bond donor with the side chain residue Tyr 237 (1.42 Å) at a strength of 32.49%, as well as the presence of arene hydrogen interaction between the center of benzene ring of benzothiazole moiety and the side chain residue Ala 241. In addition, there were hydrophobic interactions between nitrogen atom and C-5,6,7 of indole moiety with various amino acid residues: Gly 179, Ile 180, Leu 181, Ala 182, Leu 214, Val 215, His 218, Glu 219, Pro 232, Gly 233, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241, Arg 243 and Zn 281 (see supplementary information Figure S59).

### 2.3.3 Docking of compound 2b into MMP-8 active site

Docking of compound **2b** into MMP-8 active site exhibited the presence of hydrogen bond interaction between the sulfur atom and the side chain residue Tyr 237 (1.37 Å) at a strength of 26.14%, besides the presence of two arene hydrogen interactions between the center of benzene and thiazole rings in benzothiazole moiety with the side chain residues Ala 241 and Tyr 240, respectively. In addition, there were hydrophobic interactions involving C-5,6 of indole moiety and with various amino acid residues: Ile 180, Leu 181, Ala 182, Leu 214, Val 215, His 218, Glu 219, Pro 232, Gly 233, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241, Arg 243 and Tyr 248 (see supplementary information Figure S60).

### 2.3.4 Docking of compound 3 into MMP-8 active site

Docking of compound **3** into MMP-8 active site displayed the presence of a hydrogen bond interaction between the nitrogen atom of $NHCOCH_3$ group and the side chain residue Ala 182 (1.14 Å) at a strength of 3.83%. Moreover, it revealed the presence of arene interaction between the phenyl ring of acetanilide moiety and the side chain residue His 218, also arene hydrogen interactions between the centers of indole moiety with the side chain residue Ala 241. In addition, there were hydrophobic interactions involving methyl group and other atoms of the compound with various amino acid residues: Ile 180, Leu 181, Ala 182, His 183, Leu 214, Val 215, His 218, Glu 219, His 228, Pro 232, Gly 233, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241 and Arg 243 (see Supplementary information Figure S61).

### 2.3.5 Docking of compound 4 into MMP-8 active site

Docking of compound **4** into MMP-8 active site showed the presence of three arene hydrogen interactions between pyrrole ring of indole moiety and diphenyl rings of amine moiety with the side chain residues Ala 184, Leu 181 and Asn 239, respectively. Additionally, there were several hydrophobic interactions involving amino function,

C-4,5,6,7 of indole moiety, methyl groups and other atoms of the compound with various amino acid residues: Asn 178, Gly 179, Ile 180, Leu 181, Ala 182, His 183, Ala 184, Tyr 210, His 218, Glu 219, His 222, His 228, Pro 238, Asn 239 and Tyr 240 (see Supplementary information Figure S62).

#### 2.3.6 Docking of compound 5 into MMP-8 active site

Docking of compound **5** into MMP-8 active site revealed the presence of arene hydrogen interaction between the center of benzene ring of indole moiety and the side chain residue Ala 241. In addition, there were several hydrophobic interactions involving other atoms of the compound with the following amino acid residues: Gly 179, Ile 180, Leu 181, Ala 182, His 183, Val 215, His 218, Glu 219, His 222, His 228, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239 and Tyr 240 (see Supplementary information Figure S63).

#### 2.3.7 Docking of compound 6a into MMP-8 active site

Docking of compound **6a** into MMP-8 active site exhibited the presence of arene hydrogen interaction between the center of phenyl ring of benzoic acid moiety and the side chain residue Leu 181. Besides, there were several hydrophobic interactions involving other atoms of the molecule with the following amino acid residues: Asn 178, Gly 179, Ile 180, Leu 181, Ala 182, Tyr 210, His 218, Glu 219, His 222, His 228, Pro 238 and Tyr 240 (See supplementary information Figure S64).

#### 2.3.8 Docking of compound 6b into MMP-8 active site

Docking of compound **6b** into MMP-8 active site displayed two arene hydrogen interactions between the centers of indole moiety with the side chain residue Ala 241. Furthermore, it revealed the presence of arene interaction between the phenyl ring of methyl benzoate moiety and amino acid residue His 218. In addition, there were hydrophobic interactions between methyl group and other atoms of the compound with the following amino acid residues: Ile 180, Ala 182, His 183, Val 215, His 218, Pro 232, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241 and Arg 243 (see Supplementary information Figure S65).

#### 2.3.9 Docking of compound 7 into MMP-8 active site

Docking of compound **7** into MMP-8 active site showed the presence of hydrogen bond interaction between nitrogen atom of indole-3-carboxaldehyde moiety and the amino acid residue Glu 219 (0.96 Å) at a strength of 26.96%. Moreover, it revealed the presence of arene hydrogen interactions between two indole moieties with the side chain residues, Leu 181, Leu 235, Tyr 240 and Ala 241. In addition, there were hydrophobic interactions between C-4,5,6,7 with the following amino acid residues: Gly 179, Ile 180, Leu 181, Ala 182, Leu 214, Val 215, His 218, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241, Arg 243 and Tyr 248 (see Supplementary information Figure S66).

#### 2.3.10 Docking of compound 8 into MMP-8 active site

Docking of compound **8** into MMP-8 active site exhibited the presence of arene hydrogen interactions between two indole moieties with the side chain residues, Leu 181 and Leu 235. In addition, there were hydrophobic interactions between C-4,5,6,7 with the following amino acid residues: Ile 180, Leu 181, Ala 182, Leu 214, Val 215, His 218, Glu 219, Pro 232, Gly 233, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241, Arg 243 and Tyr 248 (see Supplementary information Figure S67).

#### 2.3.11 Docking of compound 9 into MMP-8 active site

Docking of compound **9** into MMP-8 active site revealed the presence of hydrogen bond interaction between nitrogen atom of indole moiety with the amino acid residue Ala 182 (0.69 Å) at a strength of 18.98%. Besides, it revealed the presence of arene hydrogen interactions in one indole moiety with the side chain residues Tyr 240 and Ala 241. In addition, there were hydrophobic interactions between C-2,3,4,7a with the following amino acid residues: Ile 180, Leu 181, Ala 182, Leu 214, Val 215, His 218, Glu 219, His 228, Ala 234, Leu 235, Tyr 237, Pro 238, Asn 239, Tyr 240, Ala 241 and Arg 243 (see Supplementary information Figure S68).

# 3 Experimental

## 3.1 General

Melting points were determined on a BÜCHI Melting Point B-540 apparatus (BÜCHI, Germany). NMR spectra ($^{1}$H NMR, $^{13}$C NMR, COSY, HMQC and HMBC) were measured on Bruker Avance DRX 500 MHz (125 MHz for $^{13}$C NMR) spectrometer (Bruker BioSpin, Billerica, MA, USA) with TMS as internal standard. Mass spectrometry experiments

were performed using a Fourier Transform Ion Cyclotron Resonance (FTICR 4.7T) mass spectrometer APEX II (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 7.0-T, 160-mm bore superconducting magnet (Bruker Analytic GmbH-Magnetics, Karlsruhe, Germany) and infinity cell and interfaced to an external (Nano) ESI or MALDI ion source. EI-MS was recorded on a Finnigan MAT 95 spectrometer (70 eV) (Waters, Milford, MA, USA) with perfluorokerosene as the reference substance for HR-EI-MS. The microwave-assisted reactions (Matthews, NC, USA) were carried out in a CEM Discover, single-mode cavity with focused MW heating (MW power supply of 0–300 W, IR temperature sensor, open or closed vessel mode, pressure range of 0–20 bar, 10-ml or 80-ml vials). Starting materials, reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The purity of the synthesized compounds was investigated by TLC, performed on Merck precoated silica gel 60 $F_{254}$ aluminum sheets with solvent mixture of dichloromethane-methanol (95-5) as eluent. Spots were visualized under UV lamp at 254 and 366 nm and again after spraying with anisaldehyde/$H_2SO_4$ reagent and heating to accelerate the reaction.

## 3.2 Synthesis

**Thermal method**: A solution of indole-carboxaldehyde (**1a–c**) (1 mmol) in methanol (10 ml) containing two drops of acetic acid (triethylamine instead of acetic acid for compound **4**) was heated at 70 °C for 5 min. A solution of primary amine (2-amino-6-ethoxybenzothiazole, 2-amino-6-methoxybenzothiazole, 4-aminoacetanilide, 4,4′-diamino-3,3′-dimethoxybiphenyl-dihydrochloride, benzophenone hydrazone, 4-aminobenzoic acid, methyl-4-aminobenzoate and 5-aminoindole) (1 mmol) in methanol (5 ml) was added slowly and the reaction mixture was refluxed at 100 °C for a given time (Table S2). After completion (TLC), evaporation of the solvent under reduced pressure furnished crude solids, which were washed with DCM then warm methanol three times and dried to afford the target compounds **2–9** with 65–89% yield (Table S2).

**Microwave synthesis**: A mixture of indole-carboxaldehyde (**1a–c**) (1 mmol) and amine (1 mmol) in methanol (2 ml) in the presence of two drops of acetic acid (triethylamine instead of acetic acid for compound **4**) was placed into a 10-ml vial with a stirring bar then stirred at room temperature for 2 min and irradiated by microwave for a given time (Table S2) at 100 °C with power of 50 W. The progress of the reaction was monitored by TLC. After completion of the reaction, the obtained solid was filtered, washed with DCM then warm methanol once time and dried to afford the title compound **2–9** with good purity (TLC) and excellent yield 79–99% yield (Table S2). The observed physical properties of title compounds **2–9** and the comparison between thermal method and microwave (MW) are given in Tables S1 and S2.

### 3.2.1 3-[(6-Ethoxybenzothiazol-2-imino)methyl]indole (2a)

$^1$H and $^{13}$C NMR data (see Table S3); MS ((+)-ESI) *m/z*: 322 $[M+H]^+$; ((−)-ESI) *m/z*:320 $[M-H]^-$; MS (HR-EI) *m/z*: 321.09200 (Calcd. 321.09303 for $C_{18}H_{15}N_3OS$ $[M]^+$).

### 3.2.2 3-[(6-Methoxybenzothiazol-2-imino)methyl] indole (2b)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 12.23 (br s, 1H, NH), 9.20 (s, 1H, CH=N), 8.38–8.34 (m, 2H), 7.75 (d, $J$=8.9 Hz, 1H), 7.59 (d, $J$=2.6 Hz, 1H), 7.55 (d, 1H), 7.34–7.26 (m, 2H), 7.06 (dd, $J$=8.9, 2.6 Hz, 1H), 3.83 (s, 3H, $OCH_3$); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 171.4 (C-2′), 160.8 (CH=N), 157.2 (C-6′), 146.3, 138.7, 138.0, 135.1, 125.01, 124.1, 122.8, 122.4, 122.4, 115.6, 114.8, 113.0, 105.6, 56.1($OCH_3$); MS ((+)-ESI) *m/z* (%): 308 $[M+H]^+$ (91), 330 $[M+Na]^+$ (43);((−)-ESI) *m/z*: 306 $[M-H]^-$; MS (HR-EI) *m/z*: 307.07586 (Calcd. 307.07738 for $C_{17}H_{13}N_3OS$ $[M]^+$).

### 3.2.3 3-[(4-Acetylaminophenylimino)methyl]indole (3)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 11.74 (br d, $J$=3.1 Hz, 1H, NH-indole), 9.95 (d, $J$=1.7 Hz, 1H, NH-acetanilide), 8.72 (s, 1H, CH=N), 8.39 (d, $J$=7.7 Hz, 1H), 7.98 (d, $J$=2.8 Hz, 1H), 7.66–7.59 (m, 2H), 7.48 (d, $J$=8.0 Hz, 1H), 7.25–7.15 (m, 4H), 2.06 (s, 3H, $COCH_3$); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 168.4 (C=O), 154.6 (CH=N), 148.6, 137.6, 136.8, 133.6, 125.2, 123.2, 122.3, 121.4, 121.2, 120.2, 115.6, 112.4, 24.4 ($CH_3$); MS ((+)-ESI) *m/z* (%): 278 $[M+H]^+$ (100), 300 $[M+Na]^+$ (99.5); ((−)-ESI) *m/z*: 276 $[M-H]^-$; MS (HR-EI) *m/z*: 277.11976 (Calcd. 277.12096 for $C_{17}H_{15}N_3O$ $[M]^+$).

### 3.2.4 3-{[(4′-Amino-3,3′-dimethoxy-1,1′-biphenyl)-4-imino]methyl}indole (4)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 12.28 (br s, 1H, NH), 9.93 (s, 1H, CH=N), 9.34 (d, $J$=11.7 Hz, 1H), 9.22 (s,

1H), 8.35 (s, 1H), 8.29 (d, $J$ = 3.1 Hz, 1H), 8.11–8.06 (m, 1H), 7.55–7.49 (m, 1H), 7.49–7.39 (m, 4H), 7.32 (dd, $J$ = 8.1, 1.9 Hz, 1H), 7.29–7.18 (m, 2H), 4.09 (d, $J$ = 18.2 Hz, 3H, $OCH_3$), 4.00 (d, $J$ = 14.2 Hz, 3H, $OCH_3$); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 158.1 (CH = N), 152.6, 152.1, 138.9, 137.5, 124.6, 124.2, 123.8, 122.6, 121.2, 120.0, 119.6, 118.6, 114.3, 112.9, 111.4, 111.1, 57.0($OCH_3$), 56.6 ($OCH_3$); MS ((+)-ESI) *m/z*: 372 $[M+H]^+$; MS (HR-EI)*m/z*: 371.16153 (Calcd. 371.16283 for $C_{23}H_{21}N_3O_2$ $[M]^+$).

### 3.2.5 3-{[(Diphenylmethylene)hydrazineylidene]methyl} indole (5)

$^1$H NMR (acetonitrile-$d_3$, 500 MHz) $\delta$ (ppm): 9.76 (br s, 1H, NH), 8.84 (s, 1H, CH = N), 7.77–7.73 (m, 2H), 7.71 (dd, $J$ = 6.9, 1.2 Hz, 2H), 7.53 (dd, $J$ = 5.6, 1.8 Hz, 3H), 7.48–7.44 (m, 3H), 7.42 (ddd, $J$ = 6.9, 4.4, 1.9 Hz, 3H), 7.21 (ddd, $J$ = 8.3, 7.1, 1.2 Hz, 1H), 6.99 (td, $J$ = 7.5, 7.0, 1.0 Hz, 1H); $^{13}$C NMR (Acetonitrile-$d_3$, 125 MHz) $\delta$ (ppm): 165.3 (C = N), 156.6 (CH = N), 138.8, 137.6, 137.2, 132.2, 130.3, 129.9, 128.8, 128.7, 128.6, 128.1, 125.1, 123.5, 122.8, 121.2, 113.5, 112.0; MS ((+)-ESI) *m/z*: 324 $[M+H]^+$; MS (HR-EI) *m/z*: 323.14164 (Calcd. 323.14170 for $C_{22}H_{17}N_3$ $[M]^+$).

### 3.2.6 3-[(4-Carboxyphenylimino)methyl]indole (6a)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 13.22 (br s, 1H, COOH), 12.18 (br s, 1H, NH), 9.94 (s, 1H, CH = N), 8.29 (d, $J$ = 3.1 Hz, 1H), 7.68–7.65 (m, 1H), 7.65–7.63 (m, 2H), 7.52 (dt, $J$ = 8.2, 1.0 Hz, 1H), 7.29–7.19 (m, 2H), 6.61–6.56 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 165.8 (C = O), 156.6 (CH = N), 151.0, 136.8, 135.4, 129.6, 122.4, 121.8, 120.4, 119.2, 118.6, 116.5, 111.2, 110.8; MS ((+)-MALDI) *m/z*: 265 $[M+H]^+$; MS (EI) *m/z* (%): 264 ($M^+$; 100).

### 3.2.7 3-[(4-Methoxycarbonylphenylimino)methyl] indole (6b)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 12.19 (br s, 1H, NH), 9.94 (s, 1H, CH = N), 8.29 (d, $J$ = 3.1 Hz, 1H), 7.66–7.64 (m, 3H), 7.52 (dt, $J$ = 8.1, 1.0 Hz, 1H), 7.29–7.19 (m, 2H), 6.61–6.56 (m, 2H) 3.73 (s, 3H, $OCH_3$); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 166.0 (C = O), 158.7 (CH = N), 153.7, 138.9, 137.5, 131.2, 123.8, 122.6, 121.2, 120.8, 118.6, 116.4, 114.0, 113.2, 52.6 ($OCH_3$); MS ((+)-MALDI) *m/z*: 279 $[M+H]^+$; MS (HR-EI) *m/z*: 278.10429 (Calcd. 278.10498 for $C_{17}H_{14}N_2O_2$ $[M]^+$).

### 3.2.8 3-[(5-Indolylimino)methyl]indole (7)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 11.70 (br s, 1H, NH), 11.08 (br s, 1H, NH), 8.82 (s, 1H, CH = N), 8.50–8.46 (m, 1H), 8.32 (s, 1H), 8.17–8.13 (m, 1H), 8.01–7.98 (m, 1H), 7.56 (dt, $J$ = 8.0, 1.0 Hz, 1H), 7.52 (dt, $J$ = 8.1, 1.0 Hz, 1H), 7.45–7.43 (m, 1H), 7.33–7.27 (m, 2H), 6.47 (ddd, $J$ = 3.1, 2.1, 0.9 Hz, 1H); $^{31}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 153.3 (CH = N), 145.9, 138.9, 137.6, 134.6, 132.7, 128.7, 126.3, 123.9, 121.2, 121.1, 118.6, 116.2, 112.8, 112.0, 111.2, 101.8; MS ((+)-MALDI) *m/z*: 260 $[M+H]^+$; MS (EI) *m/z* (%): 259 ($M^+$; 100).

### 3.2.9 2-[(5-Indolylimino)methyl]indole (8)

$^1$H and $^{13}$C NMR data (see Table S4); MS ((+)-MALDI) *m/z*: 260 $[M+H]^+$; MS (EI) *m/z* (%): 259 ($M^+$; 79), 258 (100).

### 3.2.10 5-[(5-Indolylimino)methyl]indole (9)

$^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 11.39 (br s, 1H, NH), 11.11 (br s, 1H, NH), 8.72 (s, 1H, CH = N), 8.11–8.07 (m, 1H), 7.84 (dd, $J$ = 8.5, 1.5 Hz, 1H), 7.52 (d, $J$ = 8.5 Hz, 1H), 7.48 (d, $J$ = 2.0 Hz, 1H), 7.45–7.42 (m, 2H), 7.37 (t, $J$ = 2.7 Hz, 1H), 7.16 (dd, $J$ = 8.5, 2.0 Hz, 1H), 6.58 (ddd, $J$ = 3.0, 1.8, 0.8 Hz, 1H), 6.47 (ddd, $J$ = 2.9, 1.8, 0.8 Hz, 1H); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$ (ppm): 157.0 (CH = N), 142.6, 135.8, 132.8, 126.7, 126.5, 125.9, 124.8, 124.4, 121.1, 118.8, 114.2, 110.2, 110.0, 109.7, 100.6, 99.8; MS ((+)-ESI) *m/z*: 260 $[M+H]^+$; ((−)-ESI) *m/z*: 258 $[M-H]^-$; MS ((+)-MALDI) *m/z*: 260 $[M+H]^+$; MS (HR-EI) *m/z*: 259.10927 (Calcd. 259.11040 for $C_{17}H_{13}N_3$ $[M]^+$).

## 3.3 Biological assays

### 3.3.1 Resazurin-based cytotoxicity assay

KB 3-1 cells were cultivated as a monolayer in Dulbecco's modified Eagle medium (DMEM) with glucose (4.5 g/l), L-glutamine, sodium pyruvate and phenol red, supplemented with 10% of KB 3-1 and fetal bovine serum (FBS). Cells were maintained at 37 °C and 5.3% $CO_2$-humidified air. One day prior to the test, cells of 70% confluence were detached with 0.05%/0.02% of trypsin-ethylenediamine tetraacetic acid solution in Dulbecco's phosphate-buffered saline (DPBS) and placed in sterile 96-well plates in a density of 10,000 cells in 100-µl medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 100 µM, 50 µM, or 25 µM. The stock solutions were diluted with culture

medium (10% FBS [KB 3-1]) down to pM range and dilutions added to the wells. Each concentration was tested in six replicates. Dilution series were prepared by pipetting liquid from well to well, with the control containing the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3% $CO_2$-humidified air, 30 µl of aqueous resazurin solution (175 µM) was added to each well. The cells were incubated under the conditions mentioned above for further 5 h and fluorescence was measured ($\lambda_{ex}$ = 530 nm, $\lambda_{em}$ = 588 nm). Results are given as $IC_{50}$, calculated as average of two determinations by sigmoidal dose-response curve fitting model using Graph Pad Prism software version 4.03.

#### 3.3.2 Antibacterial agar plate diffusion assay

By using a sterile pipette, 0.2 ml of the broth culture of each test bacterium was added to a sterile petri dish containing a nutrient broth agar or trypticase soy broth layer. Sterile paper disks with diameter of 6 mm were impregnated with 20 mg/ml of pure compounds dissolved in DMSO and dried under sterile conditions, and the disks were placed on agar plates pre-inoculated with broth cultures of Gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus*, or *Staphylococcus warneri* or Gram-negative bacteria *Escherichia coli* or *Pseudomonas agarici*. Plates were incubated for 24 h at 30–37 °C, and the diameter of inhibition zones was measured by a ruler in millimeter. Gentamycin at 20 mg/ml was used as positive control for each plate with test strain and DMSO as negative control.

### 3.4 Molecular docking study

Molecular modeling study of Schiff bases **2–9** was done using MOE module (MOE 2014.09; Chemical Computing Group, Montreal, Canada) as the computational software to understand the action mode of compounds through their various interactions with the active sites of MMP-8 (PDB ID: 1MNC). The program operated on an Intel Pentium 1.6-GHz processor, 512-MB memory with windows XP operating system. All the minimizations were performed with MOE until an RMSD gradient of 0.05 K Cal/mol Å with MMFF94X force field and the partial charges were automatically calculated.

Coordinates of X-ray crystal structure of (+)-Griseofulvin bound to matrix metalloproteinase (MMP-8) enzyme were obtained from the PDB (PDB ID: 1MNC). Enzyme structures were checked for missing atoms, bonds and contacts. Hydrogen atoms were added to the enzyme structure. Water molecules and bound ligands were manually deleted. Ligand molecules were constructed using the builder molecule and were energy minimized. The active site was generated using the MOE-Alpha site finder. Dummy atoms were created from the obtained alpha spheres. Ligands were docked within MMP-8 active sites using the MOE-Dock with simulated annealing used as the search protocol and MMFF94X molecular mechanics force field for 8000 interactions. The lowest energy conformation was selected and subjected to an energy minimization using MMFF94X force field.

**Acknowledgments:** The authors are grateful to the NMR and MS Departments at Bielefeld University for the spectral measurements. We would like to thank Carmela Michalek for the biological activity testing and Marco Wißbrock with Anke Nieß for the technical assistance. This research work has been financed by the German Academic Exchange Service (DAAD) with funds from the German Federal Foreign Office in the frame of the Research Training Network "Novel Cytotoxic Drugs from Extremophilic Actinomycetes" (Project ID 57166072).

## References

1. Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2011;28:196–268.
2. Gul W, Hamann MT. Indole alkaloid marine natural products: an established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. Life Sci 2005;78:442–53.
3. Sugiyama Y, Ito Y, Suzuki M, Hirota A. Indole derivatives from a marine sponge-derived yeast as DPPH radical scavengers. J Nat Prod 2009;72:2069–71.
4. Bao B, Zhang P, Lee Y, Hong J, Lee CO, Jung JH, et al. Monoindole alkaloids from a marine sponge *Spongosorites* sp. Mar Drugs 2007;5:31–9.
5. Shaaban M, Maskey RP, Wagner-Doebler I, Laatsch H. Pharacine, a natural *p*-cyclophane and other indole derivatives from Cytophaga sp. strain AM13.1 J Nat Prod 2002;65:1660–3.
6. Shaaban M, Abdel-Aziz MS. Benhamycin. Novel alkaloid from terrestrial Streptomyces sp. Nat Prod Res 2007;21:1205–11.
7. El-Sawy ER, Bassyouni FA, Abu-Bakr SH, Rady HM, Abdlla MM. Synthesis and biological activity of some new 1-benzyl and 1-benzoyl 3-heterocyclic indole derivatives. Acta Pharm 2010;60:55–71.
8. George S, Waran MP, Chakraborty A, Ravi TK. Synthesis and evaluation of the biological activities of some 3-[5-(6-methyl-4-aryl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl]-imino-1,3-di-hydro-2H-indol-2-one derivatives. Acta Pharm 2008;58:119–29.
9. Guerra AS, Malta DJ, Laranjeira LP, Maia MB, Colaço NC, Lima MC, et al. Tumor- and organ-dependent infiltration by myeloid-derived suppressor cells. Int Immunopharmacol 2011;11:816–26.

10. Mandour AH, El-Sawy ER, Shaker KH, Mustafa MA. Synthesis, anti-inflammatory, analgesic and anticonvulsant activities of 1,8-dihydro-1-aryl-8-alkyl pyrazolo (3,4-b)indole. Acta Pharm 2010;60:73–88.
11. Estevao MS, Carvalho LC, Ribeiro D, Couto D, Freitas M, Gomes A, et al. Antioxidant activity of unexplored indole derivatives: synthesis and screening. Eur J Med Chem 2010;45:4869–78.
12. Suzen S, Buyukbingol E. Anti-cancer activity studies of indolalthiohydantoin (PIT) on certain cancer cell lines. Farmaco 2000;55:246–8.
13. Mor M, Silva C, Vacondio F, Plazzi PV, Bertoni S, Spadoni G, et al. Indole based analogs of melatonin: in vitro antioxidant and cytoprotective activities. J Pineal Res 2004;36:95–102.
14. El-Sawy ER, Mandour AH, Khaled M, Islam IE, Abo-Salem HM. Synthesis, antimicrobial and anti-cancer activities of some new N-ethyl, N-benzyl and N-benzoyl-3-indolyl heterocycles. Acta Pharm 2012;62:157–79.
15. El-Sawy ER, Mandour AH, El-Hallouty SM, Shaker KH, Abo-Salem HM. Synthesis, antimicrobial and anticancer activities of some new N-methylsulphonyl and N-benzenesulphonyl-3-indolyl heterocycles. Arabian J Chem 2013;6:67–78.
16. Wu YS, Coumar MS, Chang JY, Sun HY, Kuo FM, Kuo CC, et al. Synthesis and evaluation of 3-aroylindoles as anticancer agents: metabolite approach. J Med Chem 2009;52:4941–5.
17. Pojarova M, Kaufmann D, Gastpar R, Nishino T, Reszka P, Bednarskib PJ, et al. [(2-Phenylindol-3-yl) methylene] propanedinitriles inhibit the growth of breast cancer cells by cell cycle arrest in G(2)/M phase and apoptosis. Bioorgan Med Chem 2007;15:7368–79.
18. Buyukbingol E, Suzen S, Klopman G. Studies on the synthesis and structure activity relationships of 5-(30-indolal)-2-thiohydantoin derivatives as aldose reductase enzyme inhibitors. Farmaco 1994;49:443–7.
19. Suzen S, Buyukbingol E. Evaluatwion of anti-HIV activity of 5-(2-phenyl-30-indolal)-2-thiohydantoin. Farmaco 1998;53:525–7.
20. Gu XH, Wan XZ, Jiang B. Syntheses and biological activities of bis(3-indolyl)thiazoles, analogues of marine bis(indole) alkaloid nortopsentins. Bioorg Med Chem Lett 1999;9:569–72.
21. Madadi NR, Penthala NR, Janganati V, Crooks PA. Synthesis and anti-proliferative activity of aromatic substituted 5-((1-benzyl-1H-indol-3-yl) methylene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione analogs against human tumor cell lines. Bioorg Med Chem Lett 2014;24:601–3.
22. El-Sawy ER, Abo-Salem HM, Yahya SM, Ebaid MS, Mandour AH. Synthesis, cytotoxic, pro-apoptotic evaluation and molecular docking study of some new N-substituted sulphonyl-3-indolyl heterocycles. Egypt Pharm J 2015;14:15–29.
23. Lakshmi NV, Thirumurugan P, Noorulla KM, Perumal PT. $InCl_3$ mediated one-pot multicomponent synthesis, antimicrobial, antioxidant and anti-cancer evaluation of 3-pyranyl indole derivatives. Bioorg Med Chem Lett 2010;20:5054–61.
24. Jin G, Lee S, Choi M, Son S, Kim GW, Oh JW, et al. Chemical genetics-based discovery of indole derivatives as HCV NS5B polymerase inhibitors. Eur J Med Chem 2014;75:413–25.
25. Gümrükçü G, Karaoglan GK, Erdogmus A, Gül A, Avcıata U. A novel phthalocyanine conjugated with four salicylideneimino complexes: photophysics and fluorescence quenching studies. Dyes Pigments 2012;95:280–9.
26. Gupta KC, Sutar AK, Lin CC. Polymer-supported Schiff base complexes in oxidation reactions. Coor Chem Rev 2009;253:1926–46.
27. Lorcya D, Belleca N, Fourmiguea M, Avarvari N. Tetrathiafulvalene-based group XV ligands: synthesis, coordination chemistry and radical cation salts. Coor Chem Rev 2009;253:1398–438.
28. Ashraf MA, Mahmood K, Wajid A. Synthesis, characterization and biological activity of schiff bases. International Conference on Chemistry and Chemical Process. IPCBEE, Singapore, 2011;10:1–7.
29. Przybylski P, Huczynski A, Pyta K, Brzezinski B, Bartl F. Biological properties of Schiff bases and azo derivatives of phenols. Curr Org Chem 2009;13:124–48.
30. Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4′-chlorophenyl)thiazol-2-yl] thiosemicarbazide. Eur J Pharma Sci 1999;9:25–31.
31. Awantu AF, Lenta BN, Bogner T, Fongang YF, Ngouela S, Wansi JD, et al. Dialiumoside, an Olean-18-ene Triterpenoid from Dialium excelsum. Z Naturforsch 2011;66b:624–8.
32. Sammet B, Bogner T, Nahrwold M, Weiss C, Sewald N. Approaches for the synthesis of functionalized cryptophycins. J Org Chem 2010;75:6953–60.
33. Furtado GL, Medeiros AA. Single-disk diffusion testing (Kirby-Bauer) of susceptibility of proteus mirabilis to chloramphenicol: significance of the intermediate category. J Clin Microbiol 1980;12:550–3.
34. Vilar S, Cozza G, Moro S. Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. Curr Topics Med Chem 2008;8:1555–72.
35. Zhou S, Klaunig JE. Interplay between MMP-8 and TGF-β1 and its role in regulation of epithelial to mesenchymal transition in hepatocellular carcinoma. Transl Cancer Res 2016;5:S1135–8.
36. Decock J, Hendrickx W, Thirkettle S, Gutiérrez-Fernández A, Robinson SD, Edwards DR, et al. Pleiotropic functions of the tumor- and metastasis-suppressing matrix metalloproteinase-8 in mammary cancer in MMTV-PyMT transgenic mice. Breast Cancer Res 2015;17:1–13.

**Supplemental Material:** The online version of this article (DOI: 10.1515/znc-2017-0025) offers supplementary material, available to authorized users.