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Original article

Design, synthesis and docking studies of new furobenzopyranones and pyranobenzopyranones as photoreagent towards DNA and as antimicrobial agents

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ABSTRACT

A number of new furobenzopyranones and pyranobenzopyranones carrying an electron- withdrawing function at the position 3 are synthesized in order to obtain new photoreagents towards DNA. Our interest in this study is to investigate the effect of introduction of electron withdrawing function on the position 3 of the benzo-á-pyranone ring of linear furobenzo-á-pyranone (5,8-dimethoxypsoralen) or angular pyranobenzo-á-pyranone on the biological activity, by preparing 3-cyano, carboxylic acid, carboxylic acid ester, acid hydrazide, thiosemicarbazide, or mercaptotriazole derivatives. 5-acetyl-6-hydroxybenzofuran, and 8-acetyl-7-hydroxy-4-phenylbenzopyranone are the key starting compounds on which 3-cyano-4-methylfurobenzopyranone and 3-cyano-4-methyl pyranobenzopyranone moieties were built respectively. The photobiological activity of the newly synthesized compounds was evaluated. It looks most promising for enhancement of photoreactivity of compounds towards DNA, and a certain effect was observed in the dark determining the antimicrobial activity.

Compounds **5**, **6**, **7**, **13**, **14** exhibit potential photoreactivity towards DNA, while 3-mercaptotriazole derivatives **7**, **14** possess only photosensitizing activity.

To investigate the antimicrobial data on structural basis, molecular modelling and docking studies of the tested compounds into the crystal structure of topoisomerase II DNA Gyrase B complexed with the natural inhibitor bearing the coumarin moiety clorobiocin (1kzn), using Molsoft ICM 3.4-8C program was performed in order to predict the affinity and orientation of the synthesized compounds at the active site. The ICM score values and hydrogen bonds formed with the surrounding amino acids show good agreement with predicted binding affinities obtained by molecular docking studies as verified by antimicrobial screening, where compounds **5**, **6**, **13** were the most active compounds against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Compound **13** has good affinity with the receptor and forms six hydrogen bonds with Asp-73, and two bonds with Thr-165, compound **6** has ICM score value −85.66 and forms one hydrogen bond with Asp-73, and three with Thr-165, and compound **5** has ICM score value −53 but forms one hydrogen bond with Asp-73, and four bonds with Thr-165 which may reveal the potent antimicrobial activity referred to the natural antimicrobial Clorobiocin which forms two hydrogen bonds with Asp-73 and three with Thr-165.

1. Introduction

Linear furocoumarins (furobenzo-á-pyranones, psoralens) are a well-known family of natural and synthetic photosensitizing compounds which exhibit very interesting photobiological activity. Some of these are used in PUVA photochemotherapy (Psoralen plus UVA) to treat a variety of skin diseases. They are also employed in extracorporeal photochemotherapy to treat cutaneous T-cell lymphomas and as selective immunosuppressive agents for the cure of various autoimmune diseases and to prevent rejection in organ transplants [1,2]. Furthermore, psoralen derivatives are now recognized as effective antiviral agents, especially against enveloped viruses such as the herpes simplex virus or HIV-1 [3,4].

The preferred compound is 8-methoxypsoralen (8-MOP) but 5-methoxypsoralen (5-MOP) and 4,5′,8-trimethoxypsoralen (TMP) are also used. They show good antiproliferative effect due to their capability to photo damage DNA, leading to monofunctional and two different kinds of bifunctional adducts; inter-strand cross-links (ISC) [5] and DNA–protein cross-links (DPC), tying together a DNA base and a protein amino acid [6–8].

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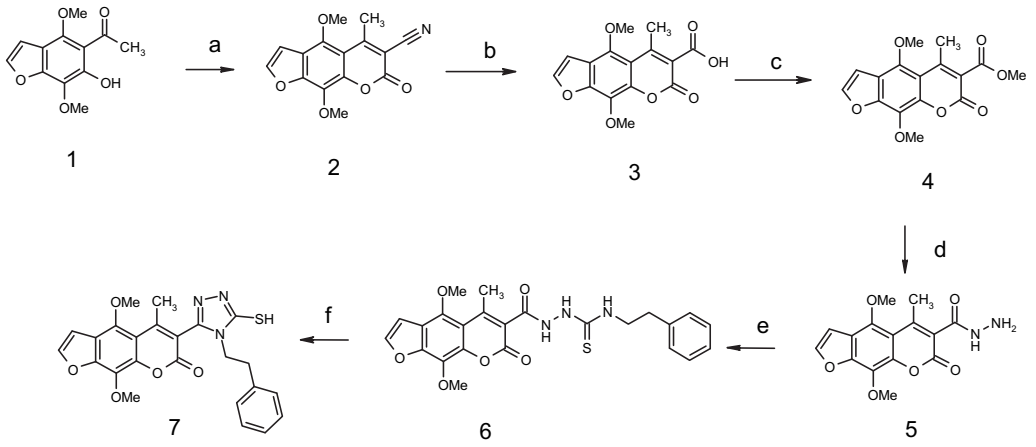


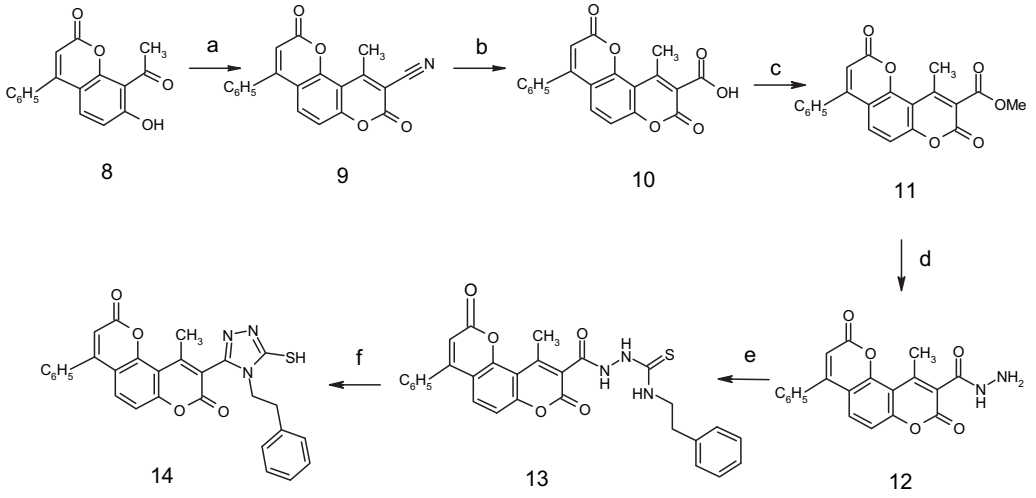
Scheme 1. Reagents, (a) ethyl cyanoacetate, sodium ethoxide, (b) Sodium hydroxide 15%, (c) thionyl chloride, methanol, (d) Hydrazine hydrate, ethanol, (e) Benzyl isothiocyanate, ethanol, (f) 2 N sodium hydroxide.

The bifunctional damage, in particular the induction of ISC, was regarded as the main cause responsible for the furocoumarin toxicity, i.e., skin erythemas, genotoxicity with induction of point mutations in bacteria [1,9–12].

Extensive photochemical and photobiological studies have been performed mainly on two series of furobenzopyranones, that is psoralens (linear furobenzopyrones) [13,14] and, angelicins (angular furobenzopyranones) [15–19]. In addition, new DNA monofunctional furobenzopyranones as 6-carbethoxy analogues [20], furobenzopyranones with modified annulations geometry [21] as well as pyridopsoralens [22–24], azapsoralens [25], furoquinolinones [26], thienoquinolinones [27] have been prepared. They prevent inter-strand cross-link formation, consequently lack skin phototoxicity [22–26], and at the same time maintain the photosensitizing activity. Also, synthesis of different series of tetrahydrobenzo- and benzofurobenzopyranone derivatives as new monofunctional DNA photobinding agents were reported [27–29].

On the other hand, in DNA replication, one group of enzymes has proved to be effective target for therapeutic agents, which is topoisomerase enzyme. DNA gyrase is a type II topoisomerase found in all bacteria and controls the topological state of DNA [30]. DNA gyrase consists of two subunits GyrA (875 amino acids) and GyrB (804 amino acids) with the active species being a heterotetramer A_2B_2 [31]. Mechanistic studies have revealed the steps involved in the gyrase supercoiling reaction [32]. This process involves the wrapping of DNA around the A_2B_2 complex, cleavage of this DNA on both strands, and the passage of a segment of DNA through the double strand break. Relegation of the break results in the introduction of two negative supercoils. These processes require the binding and hydrolysis of ATP [33]. Inhibition of DNA gyrase blocks relaxation of supercoiled DNA, relaxation being a requirement for transcription and replication. DNA gyrase is a selective target for antibacterial agents, such as the most studied quinolone and coumarin antibiotics. Quinolone drugs (e.g. ciprofloxacin) affect the protein subunit GyrA and coumarins (benzo-á-pyranones) (e.g. novobiocin, clorobiocin) act on GyrB [34].

Owing to our interest for drug molecules able to photoreact with DNA, and their biological activity, we planned to prepare a new series of linear furobenzo-á-pyranone derived from the condensation of 3-cyano-4-methyl-2H-pyran-2-one at 5, 6 of benzofuran nucleus to give a linear geometry, and another new series of angular pyranobenzo-á-pyranone derived from the condensation of 3-cyano-4-methyl-2H-pyran-2-one at 7, 8 of benzopyranone nucleus to have an angular structure, and compare the effect of different electron withdrawing substituents on the photobiological activity towards DNA, and also observe their effects in the dark.



Scheme 2. Reagents, (a) ethyl cyanoacetate, sodium ethoxide, (b) Sodium hydroxide 15%, (c) thionyl chloride, methanol, (d) Hydrazine hydrate, ethanol, (e) Benzyl isothiocyanate, ethanol, (f) 2 N sodium hydroxide.

Table 1
Photosensitizing and antimicrobial activities of furobenzopyranone analogues **2–7** and pyranobenzopyranone analogues **9–14** are expressed in terms of zone of growth inhibition (measured in mm).

Cpd. No.	*Bacillus subtilis* g+ve		*Staphylococcus aureus* g+ve	*Escherichia coli* g−ve	*Pseudoomonas aeruginosa* g−ve	*Candida albicans*
	Before UV	After UV				
xanthotoxin (8-MOP)	–	12	–	–	–	–
tetracyclin	30		36	34	–	–
amphotericin B	–		–	–	–	30
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	12	14	–	18	–	–
6	10	12	–	–	–	–
7	–	11	–	–	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–
11	–	–	–	–	–	–
12	–	–	–	–	–	–
13	18	22	30	20	–	–
14	–	12	–	–	–	–

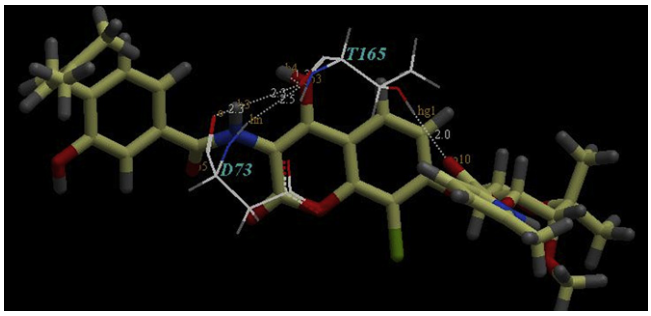


Fig. 1. Binding mode of the original ligand 1cbn (Clorobiocin) into its binding site of topoisomerase DNA gyrase B, it has ICM score −123.03, and form 10 hydrogen bonds shown as white dotted lines (Table 2), showing two hydrogen bonds between O3 (OH-4) and NH of D73 distance 2.53 A, and between NH (NHCO)and O of D73 amino acid distance 2.32 A, and another three hydrogen bonds between O-10 and HG1 of T165 distance 1.95 A, and between H-4 and O of T165 distance 1.29 A, and between H-3 and O of T165 distance 2.28 A. (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

Computer docking technique plays an important role in the drug design and discovery, as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion [35,36], and to predict the correct binding geometry for each ligand at the active site, which reveals the ICM score values and hydrogen bonds formed with the surrounding amino acids. Molsoft as flexible docking program enable us to predict favorable protein–ligand complex structures with reasonable accuracy and speed [37].

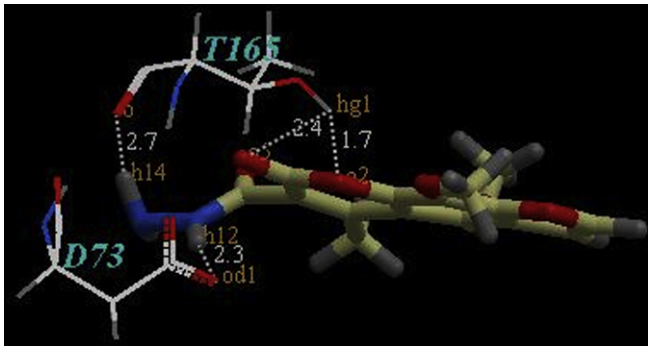


Fig. 2. Binding mode of compound **5** in the binding site of topoisomerase DNA gyrase B, it has ICM score −53.48, and form 8 hydrogen bonds shown as white dotted lines (Table 2) showing one hydrogen bond between H-12 (NH of $NHNH_2$) and OD1 of D73 amino acid distance 2.29 A, and another four hydrogen bonds between O-5 (2-oxo)and OG1 of T165 distance 2.42 A, and between O-2 (1-O-) and HG1 of T165 distance 1.71 A, and O5 (2-oxo) and NH of T165 of distance 2.57 A, and between H-14 (NH of $NHNH_2$)and O of T165 distance 2.67 A. (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

2. Chemistry

The route used for the synthesis of the new furobenzopyranone derivatives assessed in this study is shown in Scheme 1. 4,9-Dimethoxy-7-oxo-5-methyl-7H-furo [3,2-g]benzopyrane-6-carbonitrile **2** was synthesized directly, in good yield, by refluxing 1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl) ethanone **1** with ethyl cyanoacetate in sodium ethoxide [38,39]. Compound **2** was routinely hydrolyzed to the corresponding 4,9-Dimethoxy-7-oxo-5-methyl-7H-furo[3,2-g]benzopyrane-6-carboxylic acid **3** by refluxing with 15% sodium hydroxide for three hours then acidified [40]. Esterification of **3** in methanol using thionyl chloride afforded methyl 4,9-dimethoxy-7-oxo-5-methyl-7H-furo[3,2-g]benzopyrane-6-carboxylate **4** [40]. Reaction of **4** with hydrazine hydrate yielded the hydrazone **5** in good yield [19,41]. Refluxing 4,9-Dimethoxy-7-oxo-5-methyl-7H-furo[3,2-g]benzopyrane-6-carbohydrazide **5** with benzyl isothiocyanate in ethanol gave the corr esponding thiosemicarbazide **6** [42]. Finally, cyclization of 4-benzyl-1-(4,9-Dimethoxy-7-oxo-5-methyl-7H-furo[3,2-g]benzopyrane-6-

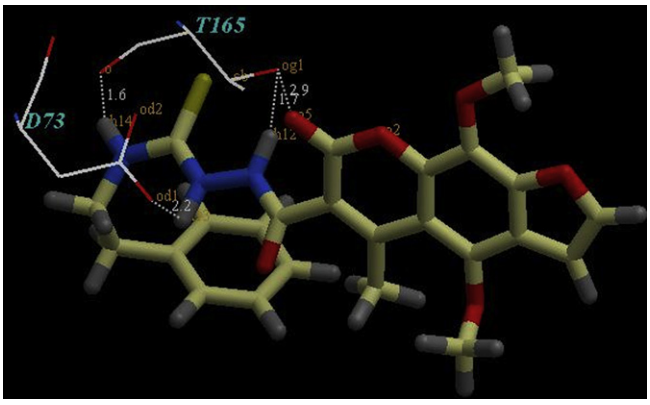


Fig. 3. Binding mode of compound **6** in the binding site of topoisomerase DNA gyrase B, it has ICM score −85.66, and form 9 hydrogen bonds shown as white dotted lines (Table 2), it shows one hydrogen bond between H13 of (CONHNHCS) and O of D73 distance 2.17 A, and and another three hydrogen bonds between O-5 (2-oxo-)and HG1 of T165 distance 2.40 A, and between H-12 (NH of CONH) and OG1 of T165 amino acid distance 1.74 A, and another bond between H-14 (NH of NHCSNHNH) and O of T165 amino acid distance 1.60 A (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

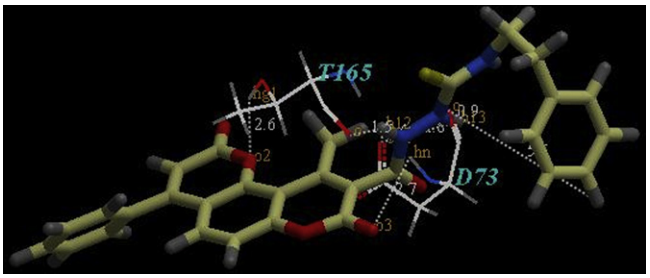


Fig. 4. Binding mode of compound **13** in the binding site of topoisomerase DNA gyrase B, it has ICM score −96.07, and form 10 hydrogen bonds shown as white dotted lines (Table 2), it shows six hydrogen bonds between O-3 (2-oxo) and NH of D73 distance 2.65 A, and H-12 (CONHNHCSNH) and OD2 of D73 distance 1.40 A, and H-13 (CONHNHCSNH) and OD2 of D73 distance 2.56 A, and H-13 (NH of NHNHCS) and O of D73 amino acid distance 0.89 A and H-12 (CONHNHCSNH) and O of D73 distance 2.39 A, and H-14(CONHNHCSNH) and O of D73 distance 2.17 A, and another two hydrogen bonds between O-2 (5-O-)and HG1 of T165 distance 2.56 A, and between H-12 (CONHNHCSNH) and O of T165 amino acid distance 1.55 A. (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

carbonyl) thiosemicarbazide **6** with 2 N sodium hydroxide afforded mercaptotriazole derivative **7** [42,43–45].

The same pathway was performed for the synthesis of pyranobenzopyranone derivatives, Scheme 2. 2(1H)-oxo-4-methyl-(2(1H)oxo-4-phenyl-pyrano)[2,3-f]benzopyran-3-carbonitrile **9** was synthesized through refluxing 8-Acetyl-7-hydroxy-4-phenyl-2H-1-benzopyran-2-one **8** with ethyl cyanoacetate in sodium ethoxide [38,39], followed by its hydrolysis to give 2(1H)-oxo-4-methyl-(2(1H)oxo-4-phenyl-pyrano)[2,3-f] benzopyran -3-car boxylic acid **10** [40]. It was then estrified to afford Methyl-2(1H)-oxo-4-methyl-(2(1H)oxo-4-phenyl-pyrano)[2,3-f] [1]benzopy ran-3-carboxylate **11** [40]. Its hydrazone derivative (1H)-oxo-4-methyl-(2(1H)oxo-4-phenyl-pyrano)[2,3-f]benzopyran-3-carbohydrazide **12** was formed by its reaction with hydrazine hydrate in ethanol [19,41]. Refuxing **12** with benzyl isothiocyanate in ethanol gave the corresponding thiosemicarbazone **13** [42]. Finally, cyclization of 4-benzyl-1-(2(1H)-oxo-4-methyl-(2(1H)oxo-4-phenyl-pyrano)[2,3-f]benzopyran-3-carbonyl) thiosemicarbazide **13** with 2 N sodium hydroxide afforded 3-(4-benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methyl-2H-(2(1H)oxo-4-phenyl-pyrano)[2,3-f]benzopyran-2-one **14** [42,43–45].

3. Results and discussion

3.1. Photosensitizing and antimicrobial screening

In this study the compounds (**2**–**7**, **9**–**14**) were screened for antimicrobial and photosensitizing activity by the disc diffusion method

Table 2
ICM Scores of Clorobiocin, the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Compounds	ICM scores	No. of hydrogen bonds	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (Å)
Clorobiocin	−123.03	10	N46….HD21	O-7	2.14
			N46….HD21	O-8	1.65
			D73……..NH	O-3 (OH-4)	2.53
			D73……..O	H-3	2.32
			I60……..NH	O-5	1.82
			E58……..Oe1	O-10	2.75
			Q72……NH	O-5	2.39
			T165…..HG1	O-10	1.95
			T165…….O	H-4	1.29
			T165…….O	H-3(OH-4)	2.28
2	−55.36	4	N46…..HD21	O-3(2-oxo)	2.15
			G77……NH	O-5(2-oxo)	1.97
			T165…..HG1	O-5(2-oxo)	2.48
			T165…..HG1	O-2(1-O-)	2.14
3	−59.21	5	N46….HD21	O-3(4-OCH3)	2.10
			G77……NH	O-5(2-oxo)	2.15
			T165…..HG1	O-5(2-oxo)	2.16
			T165…..HG1	O-2(1-O-)	2.19
			E50…….Oe2	H-12(CONH)	2.47
4	−63.09	4	N46…..HD21	O-3(4-OCH3)	2.04
			G77……..NH	O-5(2-oxo)	2.21
			T165…..HG1	O-5(2-oxo)	2.21
			T165…..HG1	O-2(1-O-)	2.13
5	−53.48	8	N46…..HD21	O-3(4-OCH3)	1.88
			T165….HG1	O-5 (2-oxo)	2.42
			T165…….O	H-14((CONHNH2)	2.67
			T165…….NH	O-5(2-oxo)	2.57
			V71……..O	H-13 (CONHNH2)	2.61
			V71……..O	H-14 (CONHNH2)	1.88
			D73….OD1	H-12(CONHNH2)	2.29
			T165…HG1	O-2 (1-O-)	1.71
6	−85.66	9	G77……..NH	O-5 (2-oxo)	1.76
			G77……..NH	O-2 (1-O-)	2.29
			R136…HH11	O-4 (OCH_3)	1.65
			R136…HH12	O-4 (OCH_3)	2.28
			R165….OG1	H-12 (CONHNHCSNH)	1.74
			T165…..HG1	O-5 (2-oxo-)	2.40
			V71…….O	H-14 (CONHNHCSNH)	2.66
			D73……..…O	H-13 (CONHNHCSNH)	2.17
			T165…….O	H-14 (CONHNHCSNH)	1.60
7	−80.48	4	N46….HD21	O-3(4-OCH3)	1.92
			G77……..NH	O-5(2-oxo)	2.49
			T165…..HG1	O-5(2-oxo)	2.66
			T165…..HG1	O-2(1-O-)	2.33

Table 2 (*continued*)

Compounds	ICM scores	No. of hydrogen bonds	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (Å)
9	−74.32	4	N46….HD21	O-4(6-oxo)	1.61
			R76….He	O-3(2-oxo)	2.47
			G77….NH	O-2(1-O-)	1.98
			R136.HH11	O-3(2-oxo)	2.41
10	−77.46	6	N46….HD21	O-4(6-oxo)	1.71
			G77……..NH	O-2(1-O-)	1.60
			R136…HH11	O-3(2-oxo)	1.76
			R136…HH11	O-5(O of OH)	2.53
			R136..HH12	O-3(2-oxo)	2.73
			R136…HH12	O-5(O of OH)	2.03
11	−79.51	5	N46…..HD21	O-4(6-oxo)	1.64
			R76……He	O-3(2-oxo)	2.21
			G77……..NH	O-2(1-O-)	1.76
			R136…HH11	O-3(2-oxo)	2.61
			R136…HH12	O-5(OCH3)	2.49
12	−70.22	7	N46…..HD21	O-4(O of CO)	1.76
			R76…..He	O-3(2-oxo)	2.56
			R76…..HH11	O-5(6-oxo)	2.76
			G77……NH	O-1 (1-O-)	1.44
			R136…HH11	O-3(2-oxo)	2.36
			R136…HH11	N-2(NH2)	2.67
			G77…….O	H-14 (CONHNHCSNH)	1.65
13	−96.07	10	D73…..…NH	O-3 (2-oxo)	2.65
			D74……..NH	O-5 (6-oxo)	2.51
			G75……NH	O-5 (6-oxo)	2.66
			T165 ….HG1	O-2 (5-O-)	2.56
			T165……O	H-12 (CONHNHCSNH)	1.55
			D73…….OD2	H-12 (CONHNHCSNH)	1.40
			D73…….OD2	H-13 (CONHNHCSNH)	2.56
			D73……..O	H-13 (CONHNHCSNH)	0.89
			D73……..O	H-12 (CONHNHCSNH)	2.39
			D73……..O	H-14 (CONHNHCSNH)	2.17
14	−93.65	1	N46…..HD21	O-4(OCH_3)	2.09

[47] compared with reference compound xanthotoxin, which can be clinically investigated. The test organism used was *Bacillus subtilis*.

Also, compounds were tested for their antimicrobial activity [48] against *Staphylococcus aureus* representatives of gram positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as representatives of gram negative bacteria and the fungus *Candida albicans*, the results are presented in Table 1.

3.2. Molecular modelling studies

To predict the antimicrobial data on a structural basis, Automated docking studies were carried out using Molsoft ICM 3.4-8C program [36], the scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the topoisomerase II DNA gyrase B enzyme. The protein–ligand complex was constructed based on the Xray structure topoisomerase II gyrase B with its bound inhibitor Clorobiocin available through the RCSB Protein Data Bank (PDB entry 1KZN) [49]. The scoring functions of the compounds were calculated from minimized ligand protein complexes.

In order to compare the binding affinity of the newly synthesized benzopyranone analogues, we docked compounds **2**–**7**, **9**–**14** into the empty binding site of topoisomerase II DNA gyrase B (1kzn), with its bound inhibitor clorobiocin, Figs. 1–4 show the docking solutions with the highest predicted binding affinity for topoisomerase II DNA gyrase B. Fig. 1b show binding mode of the original ligand into its binding site, while Figs. 2–4 show binding modes of compounds **5**, **6**, **13** respectively.

As shown from the Table 2, and Figs. 1–4 the following results can be drawn:

Clorobiocin (the original ligand) reveals ICM score of −123.03 and form two hydrogen bonds with Asp-73 and another three bonds with Thr-165 (Table 2, Fig. 1). Compound **5** exhibits relatively weak binding affinity with ICM score of −53.48 but form one hydrogen bond with Asp-73, and another four bonds with Thr-165 (Table 2, Fig. 2). Compound **6** possess ICM scores of −85.66 form one hydrogen bond with Asp-73, and another three bonds with Thr-165 (Table 2, Fig. 3). Compound **13** which is the most active compound as antibacterial agent possess ICM scores of −96.07 form six hydrogen bonds with Asp-73, and another two bonds with Thr-165 (Table 2, Fig. 4).

4. Conclusion

The data reported herein indicates that pyranobenzopyrones represents a new interesting class of potentially useful compounds as photosensitizer.

Substituted furobenzopyranones, pyranobenzopyranones represent analogues of tricyclic systems with additional substituents in position 3 that increase the conjugation of the system. Derivatives with cyano, carboxylic acid, and/or ester moieties are devoid of any activities, but substitution with acid hydrazide, thiosemicarbazide decrease ICM scores and increase affinity with the enzyme and it was found that hydrogen bonds formation with Asp-73 and Thr-165 amino acid residues may be responsible for the antibacterial activity as referred to Clorobiocin.

According to these results, we can conclude that compound **13** appears to be the most interesting compound among the newly synthesized and seem potentially attractive as photochemotherapeutic drug, and as antibacterial against gram positive and gram negative bacteria.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on Gallenkamp and Kofler melting point apparatus and are uncorrected. Elemental analyses

(C, H, N) were performed by Micro Analytical Center, Faculty of Science, Cairo University, the values were found to be within ±0.4% of the theoretical ones unless otherwise indicated. Infrared spectra were recorded on Shimadzu IR 435 Spectrophotometer or on a Genesis II FTIR TM, Mattson, 5225, Verona Road, Madison wi. 53711 USA, using KBr discs. ^{1}H NMR spectra were scanned on Varian 360 MHz and 90 MHz spectrometers (chemical shifts are given in part per million (ppm) downfield from TMS). Mass spectra were made on a Finnigan Mat 212-spectrometer (EI. 120 eV, R 1000). Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates 60-F-254 (Merck; 0.25 mm), developing with chloroform.

*5.1.1. 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbonitrile (**2**)*

A solution of 1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl) ethanone (**1**, 2.4 g, 0.01 mol) in sodium ethoxide 30 ml, was reacted with ethyl cyanoacetate (1.13 ml, 0.01 mol) by refluxing the mixture for 20 h and allowed to cool, The precipitate obtained was collected, dried and crystallized from ethanol/dioxane giving **2** as yellow powder (1.8 g, 66%), mp 254 °C. IR (KBr,cm^{-1}): 2240 (CN), 1720 (C═O), 1710 (C═O), 1500 (C═C), ^{1}H NMR (DMSO-d_6): ä 2.51 (s, 3H, CH_3), 4.05, 4.14 (s, 2 × 3H, 2 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64–7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$), MS: (*m*/*z*, %) = (285, 100). Anal. Calc. for $C_{15}H_{11}O_5N$: C, 63.16; H, 3.86: N, 4.91.Found: C, 63.50; H, 4.40; N, 4.80

*5.1.2. 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carboxylic acid (**3**)*

A solution of 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbonitrile (**2**, 2.80 g, 0.01 mol) and sodium hydroxide (150 ml, 15%) was refluxed for 3 h, after chilling, the mixture was acidified with dilute HCl, water (200 ml) was added, leave overnight. The solid was filtered and washed several times with water and dried. The crude products were crystallized from methanol. (2.3 g, 75%), m.p 137 °C. IR (KBr,cm^{-1}): 3300–2200 (OH of COOH), 1718, 1640 (2 × C═O), 1600 (NH), 1500 (C═C), ^{1}H NMR (DMSO-d_6): ä 2.51 (s, 3H, CH_3), 4.05, 4.14 (s, 2 × 3H, 2 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64–7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$), 11.50 (OH of COOH). Anal. Calc. for $C_{15}H_{12}O_7$: C, 59.21; H, 3.95.Found: C, 59.3; H, 4.00.

*5.1.3. Methyl 5, 9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carboxylate (**4**)*

To a cold solution of 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carboxylic acid (**3**, 3.1 g, 0.01 mol) in methanol, add thionyl chloride (1.12 ml, 1.2 mol) dropwise, stirr for 20 min, then reflux for 4 h, distill under reduced pressure. The separated product were collected, washed, dried and crystallized from ethanol. (2.7 g, 85%), m.p 167 °C. IR (KBr,cm^{-1}): 1720, 1690 (2 × C═O), 1620 (NH), 1550 (C═C), ^{1}H NMR (DMSO-d_6): ä 2.50 (s, 3H, CH_3), 4.05, 4.10, 4.14 (3 × s, 3 × 3H, 3 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64–7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$). Anal. Calc. for $C_{16}H_{14}O_7$: C, 60.38; H, 4.40.Found: C, 60.5; H, 4.6.

*5.1.4. 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbohydrazide (**5**)*

A solution of Methyl 5, 9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carboxylate (**4**, 3.2 g, 0.01 mol), and hydrazine hydrate 99% (1.0 ml, 0.02 mol) in ethanol 30 ml, heat under reflux for 6 h The reaction mixture was chilled and filtered. The obtained precipitate was collected, dried and recrystallized from methanol.

(2.3 g g, 75%), m.p 144 °C. IR (KBr,cm^{-1}): 3224, 3150 (NH), 1731,1683 (C═O), 1610 (NH), 1590 (C═C), ^{1}H NMR ($CDCl_3$-d_6): ä 2.51 (s, 3H, CH_3), 4.05, 4.14 (s, 2 × 3H, 2 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64–7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$), 11.20 (3 × s,3 × 1H, NH(s) exch.), MS: (*m*/*z*, %) = (318, 24.9). Anal. Calc. for $C_{15}H_{14}N_2O_6$: C, 56.60; H, 4.40; N, 8.80.Found: C, 56.4; H, 4.6; N, 8.90.

*5.1.5. 4-Benzyl-1(5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbonyl) thiosemicarbazide (**6**)*

A mixture of 5,9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbohydrazide (**5**, 3.18 g, 0.01 mol), and benzyl isothiocyanate (1.42 ml, 0.01 mol) in ethanol 20 ml, was refluxed for 2 h. After cooling, the solid was filtered, and crystallized from ethanol. (3.7 g, 80%), m.p 210 °C. IR (KBr,cm^{-1}): 3300,3150 (3 × NH), 1715−1700 (2 × C═O), 1380 (C═S), ^{1}H NMR ($CDCl_3$-d_6): ä 2.35 (s, 3H, CH_3), 4.18 (s, 2H, CH_2 of benzyl), 4.05, 4.18 (s, 2 × 3H, 2 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64-7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$), 7.45–6.65 (m, 5H, phenyl proton), 9.60, 10.30, 11.20 (3 × s, 3 × 1H, 3 × NH exch.). Anal. Calc. for $C_{23}H_{21}N_3O_6S$: C, 59.10; H, 4.49; N, 8.99.Found: C, 58.7; H, 4.5; N, 8.96.

*5.1.6. 3-(4-Benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)-5,9-Dimethoxy-4-methyl-2H-furo[3,2-g]benzopyrane-2-one (**7**)*

A solution of 4-benzyl-1-(5, 9-Dimethoxy-2-oxo-4-methyl-2H-furo[3,2-g]benzopyrane-3-carbonyl) thiosemicarbazide (**6**, 4.7 g, 0.01 mol) in 2 N sodium hydroxide (12 ml) was refluxed for 2 h. The reaction mixture was cooled, and acidified with dilute hydrochloric acid to pH 5–6. The produced precipitate was filtered and crystallized from isopropanol.

(3.2 g, 70%), m.p 287 °C. IR (KBr,cm^{-1}): 2450 (SH), 1715–1700 (2 × C═O), 1511 (C═C), ^{1}H NMR ($CDCl_3$-d_6): ä 2.35 (s, 3H, CH_3), 5.20 (s, 2H, CH_2 of benzyl), 4.05, 4.18 (s, 2 × 3H, 2 × OCH_3), 6.95–6.96 (d, 1H, H-6 of benzofuran, $J_{6,7} = 2.2$), 7.64–7.68 (d, 1H, H-7 of benzofuran, $J_{7,6} = 1.8$), 7.45–6.65 (m, 5H, phenyl proton), MS: (*m*/*z*, %) = (449, 59.5). Anal. Calc. for $C_{23}H_{19}$ N_3O_5S: C, 61.47; H, 4.23; N, 9.35.Found: C, 61.2; H, 4.0; N, 9.7.

*5.1.7. 8-Acetyl-7-hydroxy-4-phenyl-2H-1-benzopyran-2-one (**8**)*

Was previously reported [46].

*5.1.8. 2(1H)-oxo-4-Methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran-3-carbonitrile (**9**)*

A solution of 8-Acetyl-7-hydroxy-4-phenyl-2H-1-benzopyran-2-one (**8**, 2.8 g, 0.01 mol) in sodium ethoxide 30 ml, was reacted with ethyl cyanoacetate (1.13 ml, 0.01 mol) by refluxing the mixture for 20 h and allowed to cool, The precipitate obtained was collected, dried and crystallized from ethanol/dioxane giving **9** as yellow powder (2.2 g, 66%), m.p 254 °C.

IR (KBr,cm^{-1}): 2240 (CN), 1690, 1720 (2 × C═O), 1560 (C═C), ^{1}H NMR (DMSO-d_6): ä 2.51 (s, 3H, CH_3), 6.39 (s, 1H, H-7), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, 1H, H-10, $J_{10,9} = 9.9$), 7.27–7.44 (m, 5H, C_6H_5-8). Anal. Calc. for $C_{20}H_{11}NO_4$: C, 72.95; H, 3.34: N, 4.26.Found: C, 63.5; H, 4.40; N, 4.6

*5.1.9. 2(1H)-oxo-4-Methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran-3-carboxylic acid (**10**)*

A solution of (**9**, 3.3 g, 0.01 mol) and sodium hydroxide (150 ml, 15%) was refluxed for 3 h, after chilling, the mixture was acidified with dilute HCl, water (200 ml) was added, leave overnight. The solid was filtered and washed several times with water and dried. The crude products were crystallized from methanol. (2.6 g, 75%), m.p 137 °C. IR (KBr,cm^{-1}): 3300−2200 (OH of COOH), 1718, 1690,1640 (3 × C═O), 1595 (C═C), ^{1}H NMR (DMSO-d_6): ä 2.35 (s,

3H, CH_3), 6.15 (s, 1H, H-7), 7.27 (s, 5H, C_6H_5-8), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, 1H, H-11, $J_{11,10} = 9.9$), 7.44–7.57 (m, 5H, aromatic protons), 11.80 (OH of COOH). Anal. Calc. for $C_{20}H_{12}O_6$: C, 68.97; H, 3.49.Found: C, 69.2; H, 3.3.

*5.1.10. Methyl-2(1H)-oxo-4-methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran-3-carboxylate (**11**)*

To a cold solution of (**10**, 3.48 g, 0.01 mol) in methanol, add thionyl chloride (1.4 ml, 1.2 mol) dropwise stirr for 20 min, then reflux for 4 h, distill under reduced pressure. The separated product were collected, washed, dried and crystallized from ethanol. (3.0 g, 85%), m.p 167 °C. IR (KBr,cm^{-1}): 1720, 1690 (3 × C═O), ^{1}H NMR (DMSO-d_6): ä 2.51 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 6.15 (s, 1H, H-7), 7.27 (s, 5H, C_6H_5-8), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, 1H, H-10 $J_{10,9} = 9.9$), 7.44–7.57 (m, 5H, aromatic protons). Anal. Calc. for $C_{21}H_{14}O_6$: C, 69.61; H, 3.87.Found: C, 69.7; H, 3.6.

*5.1.11. 2(1H)-oxo-4-Methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran-3-carbohydrazide (**12**)*

A solution of Methyl-2(1H)-oxo-4-methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran -3-carboxylate (**11**, 3.6 g, 0.0 1 mol), and hydrazine hydrate 99% (1.0 ml, 2 mol) in ethanol 30 ml, heat under reflux for 6 h The reaction mixture was chilled and filtered. The obtained precipitate was collected, dried and recrystallized from methanol.

(2.7 g, 75%), m.p 144 °C. 3224, 3150 (NH), 1731,1683, 1670 (3 × C═O), 1600 (C═C), ^{1}H NMR ($CDCl_3$-d_6): ä 2.51 (s, 3H, CH_3), 6.39 (s, 1H, H-7), 7.27 (s, 5H, C_6H_5-8), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, 1H, H-10 $J_{10,9} = 9.9$), 7.44–7.57 (m, 5H, aromatic protons), 11.20 (s,1H, NH exch.). Anal. Calc. for $C_{20}H_{14}N_2O_5$: C, 66.30; H, 3.87; N, 7.73.Found: C, 66.5; H, 3.9; N, 7.7.

*5.1.12. 4-Benzyl-1-(2(1H)-oxo-4-methyl-8-phenyl-6(5H)-oxo-pyrano[2,3-f]benzopyran-3-carbonyl) thiosemicarbazide (**13**)*

A mixture of (**12**, 3.6 g, 0.01 mol), and benzyl isothiocyanate (1.42 ml, 0.01 mol) in ethanol 20 ml, was refluxed for 2 h. After cooling, the solid was filtered, and crystallized from ethanol. (3.1 g, 60%), m.p 210 °C. IR (KBr,cm^{-1}): 3300,3150 (3 × NH), 1715–1700 (3 × C═O), 1510 (C═C), 1380 (C═S), ^{1}H NMR ($CDCl_3$-d_6): ä 2.35 (s, 3H, CH_3), 4.05 (s, 2H, CH_2 of benzyl), 6.39 (s, 1H, H-7), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, H-10, $J_{10,9} = 9.9$), 7.45–6.65 (m, 10H, phenyl protons), 9.40, 10.30, 11.65 (3 × s, 3 × 1H, 3 × NH exch.). Anal. Calc. for $C_{28}H_{21}N_3O_5S$: C, 65.75; H, 4.11; N, 8.22.Found: C, 65.9; H, 3.9; N, 8.5.

*5.1.13. 3-(4-Benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)-4-methyl-8-phenyl-2H-(6(5H)-oxo-pyrano)[2,3-f]benzopyran-2-one (**14**)*

A solution of (**13**, 5.1 g, 0.01 mol) in 2 N sodium hydroxide (12 ml) was refluxed for 2 h. The reaction mixture was cooled, and acidified with dilute hydrochloric acid to pH 5–6. The produced precipitate was filtered and crystallized from isopropanol.

(2.5 g, 50%), m.p 287 °C. IR (KBr,cm^{-1}): 2450 (SH), 1715–1700 (2 × C═O), 1590 (C═C), ^{1}H NMR ($CDCl_3$-d_6): ä 2.35 (s, 3H, CH_3), 4.80 (t, 2H, CH_2 of benzyl), 6.31 (s, 1H, H-8), 7.04–7.08 (d, 1H, H-9, $J_{9,10} = 9.3$), 7.22–7.25 (d, H-10, $J_{10,9} = 9.9$), 7.45–6.65 (m, 10H, phenyl protons). Anal. Calc. for $C_{28}H_{19}N_3O_4S$: C, 68.15; H, 3.85; N, 8.52.Found: C, 68.3; H, 4.0; N, 8.8.

5.2. Photosensitizing activity

In this study the compounds were screened for antimicrobial and photosensitizing activity by the disc diffusion method [47] compared with reference compound as xanthotoxin, which can be clinically investigated. The test organism used was *B. subtilis*.

5.2.1. Pre-experimental procedure

a) Nutrient agar medium: 0.3% of beef extract, 0.5% of pepone, 0.1% of dipotassium hydrogen phosphate, and 1.5% agar.
b) Broth culture of the organism *B. subtilis*, was incubated overnight and then broth culture of the organism was prepared (each plate contains 15 ml of the agar medium previously seeded with 0.2 ml of 18 h growth culture in liquid media for the organism).
c) Paper discs: Whatman No.1 filter paper disc (6 mm) were sterilized and impregnated with the tested compounds 10 mg/ml dimethylformamide (DMF), and allowed to dry overnight.

5.2.2. Experimental

0.02 ml of the prepared broth culture was added carefully in the sterile Petri dishes then; 10 ml of the liquefied nutrient agar medium was added, allowed to be mixed uniformly and solidified agar layer. Each dish contains a disc impregnated with DMF (neglect effect of the solvent), and another disc impregnated with xanthtoxin 10 mg/ml DMF as reference compound.

Two groups of plates were used, one as test plate was incubated in the dark at 37 °C for 3 h before irradiation to allow for diffusion of the tested compounds through the agar layer, and the duplicate plate was left in the incubator overnight as control to determine the antimicrobial activity.

Covers were removed from the tested Petri dishes and exposed to UV lamp (365 nm) for 20 min. After irradiation, the plates were reincubated in the dark at 37 °C overnight and examined for antimicrobial and photosensitizing activities by measuring the produced inhibition zones.

5.3. Antimicrobial activity

5.3.1. Test organisms

The antimicrobial activity of compounds was evaluated in vitro against *B. subtilis*, and *S. aureus* (as representative examples of gram positive bacteria). *E. coli* and *P. aeruginosa* (as representative examples of gram negative bacteria) and the fungus *C. albicans*.

5.3.2. Method

Agar plate disc diffusion technique [48].

5.3.3. Procedures

Standards of 6 mm in diameter sterilized Whatman filter paper discs were impregnated with 50 mg/mL solution of the test compound and standard (tetracycline and amphotercin B) dissolved in DMF and allowed to air dry. The discs were applied to the surface of nutrient agar plates seeded with the test organism (each plate contains 15 ml of the agar medium previously seeded with 0.2 mL of 18 h growth culture in liquid media for each organism). The incubated plates were incubated at 37 °C for 48 h and the inhibition zone was measured in mm around each disc. Discs impregnated with DMF were used as a control. The antibacterial reference tetracycline and the antifungal reference amphotericin B were assayed concurrently.

5.4. Molecular modelling studies

5.4.1. Generation of ligand and enzyme structures

The crystal structure of target protein topoisomerase (1kzn) was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/welcome.do). All bound waters ligands and cofactors were

removed from the protein. The amino acids of the binding site where defined using data in pdbsum (http://www.ebi.ac.uk/thoronton-srv/databases/pdbsum/).

5.4.2. Preparation of small molecule

A set of 3-substituted furobenzopyrone, and pyranobenzopyrone analogues synthesized to inhibit topoisomeras II DNA gyrase B was compiled by us earlier; ChemDraw 3D structures were constructed using ChemDraw 3D ultra 8.0 software [Molecular Modelling and Analysis; Cambridge Soft Corporation, USA (2003)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with MM2, Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (*.mol).

5.4.3. Docking using Molsoft ICM 3.4-8C program

5.4.3.1. Convert our PDB file 1kzn into an ICM object. This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates. Click on MolMechanics/Convert/Protein, and then delete water molecules.

5.4.3.2. To perform ICM small molecule docking.

a) Setup docking project

i) Set Project Name: Click on Docking/Set project name, press OK.

ii) Setup the receptor

Click on Docking/Receptor Setup, enter the receptor molecule in the receptor molecule data entry box (a_*) will do, then click on identify the binding sites button to identify the potential ligand binding pockets, press OK.

After the receptor setup is complete, the program normally displays the receptor with selected binding site residues highlighted in yellow xstick presentation.

iii) Review and adjust binding site

ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residues expected to be involved in ligand binding. Click on the menu Docking/Review/Adjust ligand/Box.

vi) Make receptor maps

The step now is to construct energy maps of the environment within the docking box. Click on menu Docking/Make Receptor Maps, select the resolution of the map by entering a value into the grid cell size data entry box which is 0.5, this step takes few minutes.

b) Start docking simulation

Use interactive docking to dock one ligand at a time. Click on menu Docking/Interactive docking/Mol Table Ligand, use the drop down arrow to find the table of ligand and/or Compounds we wish to dock, and then enter the thoroughness which represent the length of simulation. Generally 1 is reasonable value, select Calc ICM Score, then select Display run which display the ligand sampling the energy in the ligand binding project.

5.4.3.3. Display the result. Click Docking/Browse/Stack Conformations.

ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved.

The mode of interaction of the 1cbn (Clorobiocin) within 1kzn was used as a standard docked model as well as for RMSD calculation. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

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