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Identification of novel pyrazole and benzimidazole based derivatives as PBP2a inhibitors: Design, synthesis, and biological evaluation

Menna-Allah W. Shalaby[a], Eman M. E. Dokla[a], Rabah A. T. Serya[a], Khaled A. M. Abouzid[*a,b]

[a]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia 11566, Cairo, Egypt*
[b]*Department of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt*

ABSTRACT

The antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) is attributable to the expression of the high molecular mass transpeptidase enzyme, penicillin-binding protein 2a (PBP2a), an enzyme that catalyzes the cross-linking reaction step in the cell wall biosynthesis in the face of the challenge by β-lactam antibiotics. In the current study, ten pyrazole and benzimidazole based-compounds were designed, synthesized, and evaluated as anti-MRSA agents. These derivatives were screened for their antibacterial activity against two *Staphylococcus* (*S.*) *aureus* strains; methicillin-sensitive *Staphylococcus aureus* (MSSA) ATTC6538 and MRSA USA300 strains. Three of the tested compounds **(XII, XIII, and XIV)** exhibited moderate bactericidal activity against MSSA, MRSA, and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains. Docking of these compounds into the allosteric site of PBP2a showed comparable binding modes to that of the lead quinazolinone PBP2a inhibitors suggesting a similar mode of action. The present study presents a promising candidate for further optimization as a potential PBP2a inhibitor targeting MRSA infection.

Keywords: *Pyrazoles; benzimidazoles; resistance; penicillin-binding protein 2a inhibitors; anti-MRSA agents*

***Correspondence** | Khaled A. M. Abouzid; Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia 11566, Cairo, Egypt. Email: khaled.abouzid@pharma.asu.edu.eg

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1. INTRODUCTION

Despite the extensive efforts directed toward developing effective therapies to fight antimicrobial resistance in the past two decades, it remains a threatening challenge throughout the world. MRSA is an antibiotic-resistant strain of *S. aureus* first isolated in 1961, one year after the introduction of methicillin, the first penicillinase-resistant semisynthetic penicillin **[1]**. Thereafter, MRSA has become the major cause of hospital and nosocomial acquired infections imposing a serious economic cost on patients and hospitals **[2]**.

The broad resistance of MRSA to the β-lactam class is related to the properties of the key component of this resistance mechanism, the "acquired" penicillin-binding protein 2a, that has an intrinsic reduced susceptibility to β-lactam inactivation. The basis for its reduced affinity is its maintenance for a closed active-site

conformation that is regulated by allostery **[3]**. PBP2a is the gene product of *mecA,* a gene cassette that is not native to *S. aureus* but it has been acquired from unknown sources **[4]**. PBP2a can perform the critical cell wall cross-linking reaction even in the presence of β-lactam antibiotics, meanwhile, the other four native PBPs are inhibited **(Fig. 1) [5]**. Ceftaroline, an approved β-lactam antibiotic, has an exceptional affinity for PBP2a due to its ability to bind noncovalently to the allosteric site that predisposes the enzyme to inhibition by a second molecule of antibiotic at 60 A^{o} distance **[6,7]**. Also, ceftobiprole is another cephalosporin β-lactam antibiotic that exhibits good anti-MRSA activity by inhibiting its antibiotic resistance determinant PBP2a **[8]**. This new anti-MRSA β-lactam owes its effectiveness to the increased hydrophobic interaction of the R_2 substituent with PBP2a active site. However, resistance to these two antibiotics has already emerged **[9]**.

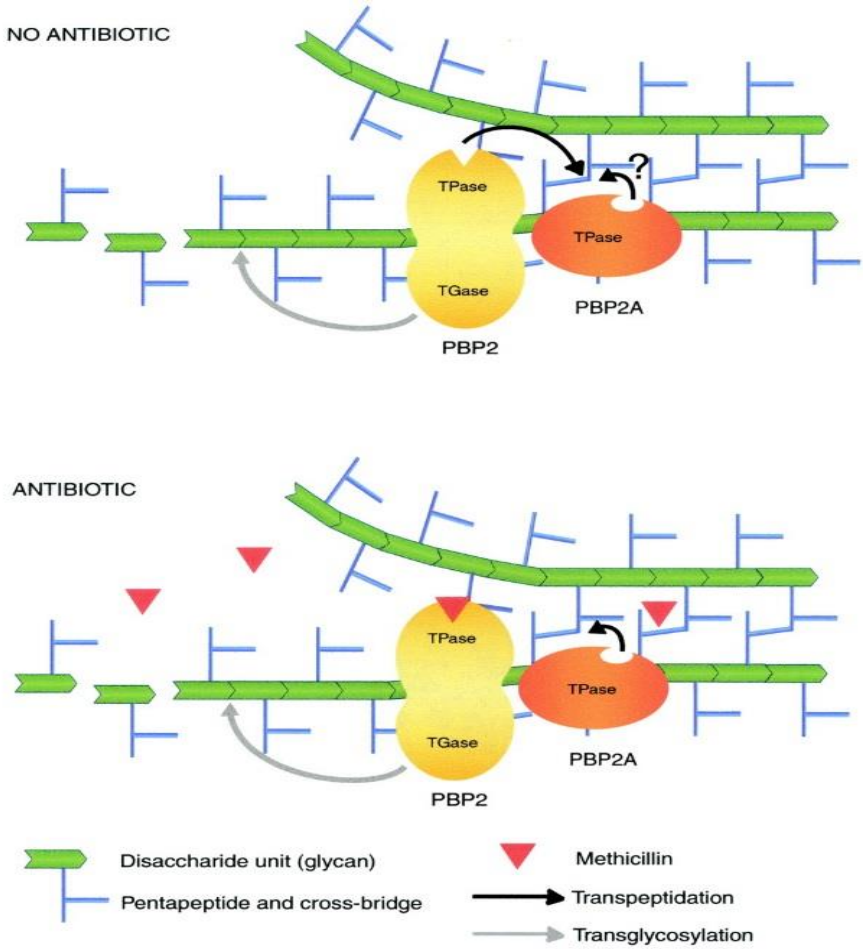


Fig. 1. Mechanism of MRSA resistance to β-lactam antibiotics**.** Top Row**:** In the absence of antibiotic it is assumed that all the penicillin-binding protein enzymes (PBP2 and PBP2A that presents in MRSA strains) participate in the crosslinking of staphylococcal peptidoglycan. Bottom Row: When Methicillin is added to the medium, β-lactam antibiotics permanently inactivate PBP2 enzymes, which are essential for bacterial life, by permanently binding to their active site. However**,** (PBP2a) which has an altered active site that will not allow β-lactam antibiotics to bind, remains functional and continues cell wall crosslinking resulting in resistance to this entire subclass of antibiotics.

Hence, there is an urgent need for the development of new antibiotics targeting PBP2a. This strategy has been adopted in the last few years yielding several small molecules such as oxadiazoles, quinazolinones and other new non-covalent inhibitors **(Fig. 2) [10–13]**. Here we disclose novel pyrazole and benzimidazole-based small molecules through bioisosteric modifications of the lead compound; quinazolinone, while introducing new scaffolds having the same pharmacophoric features. Three compounds exhibited moderate antibacterial activity against vancomycin and linezolid-resistant MRSA, providing potential leads for further optimization as novel antimicrobials against MRSA infection.

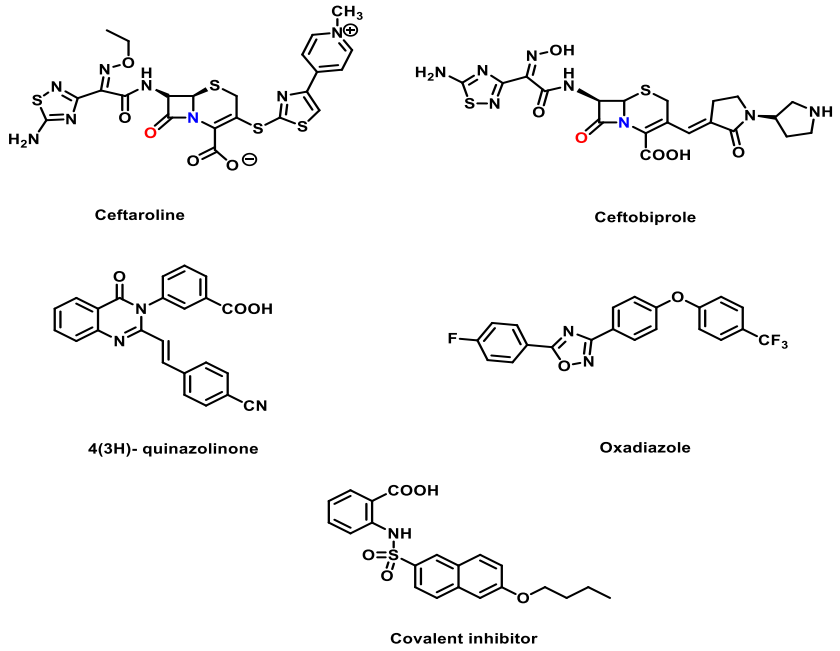


Fig. 2. Chemical structures of reported small molecules targeting PBP2a via different mechanisms.

2. Rationale and Design

In 2015, Bouley *et al* reported a novel quinazolinone-based small molecule, which was effective as a non-β-lactam antibiotic against MRSA pathogen through targeting the penicillin-binding protein 2a (PBP2a) thus inhibiting the cell wall biosynthesis **[11]**. Till today, scaffolding is a common optimization approach, so we decided to replace the quinazolinone scaffold by different bioisosteric heterocyclic scaffolds such as (pyrazole and benzimidazole) while maintaining the same pharmacophoric features of the lead compound **(Fig. 3, 4)**. We aimed to maintain the chalcone and the substituted phenyl in the correct orientation to allow complementary interactions with the allosteric site of the PBP2a. Also, we were aiming to explore the SAR of these compounds with different derivatives in both positions, by replacing the cyanostyrene and the benzoic acid parts in a manner that could attain the same binding mode of the lead compound. Our efforts resulted in the development of new pyrazole (**Scheme 1**) and benzimidazole (**Scheme 2**) derivatives with moderate activity against vancomycin and linezolid-resistant MRSA.

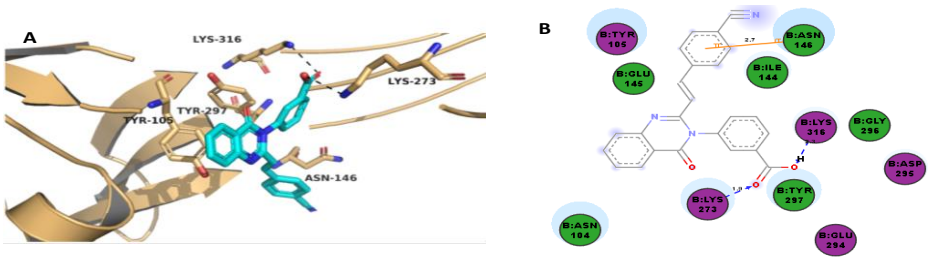


Fig. 3. 3D and 2D binding mode of the quinazolinone-based lead compound

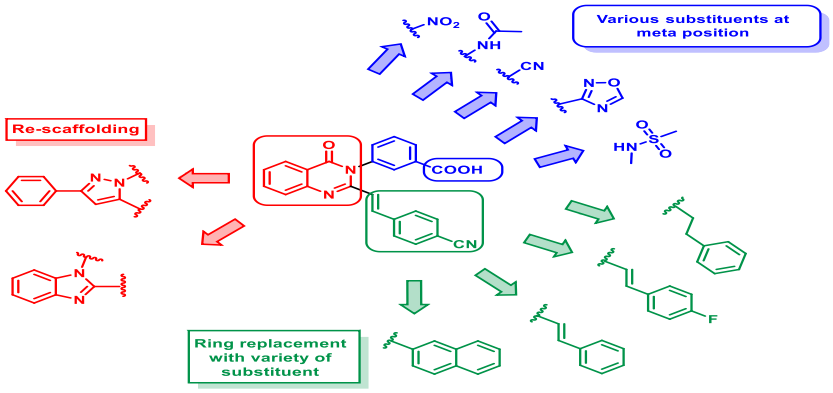


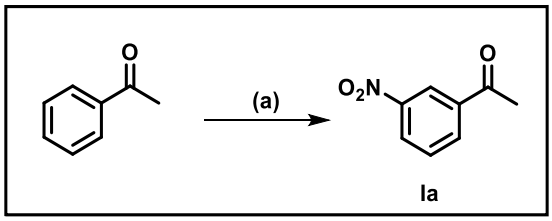
Fig. 4. Rescaffolding and optimization strategy of the quinazolinone-based lead compound

3. RESULTS AND DISCUSSION

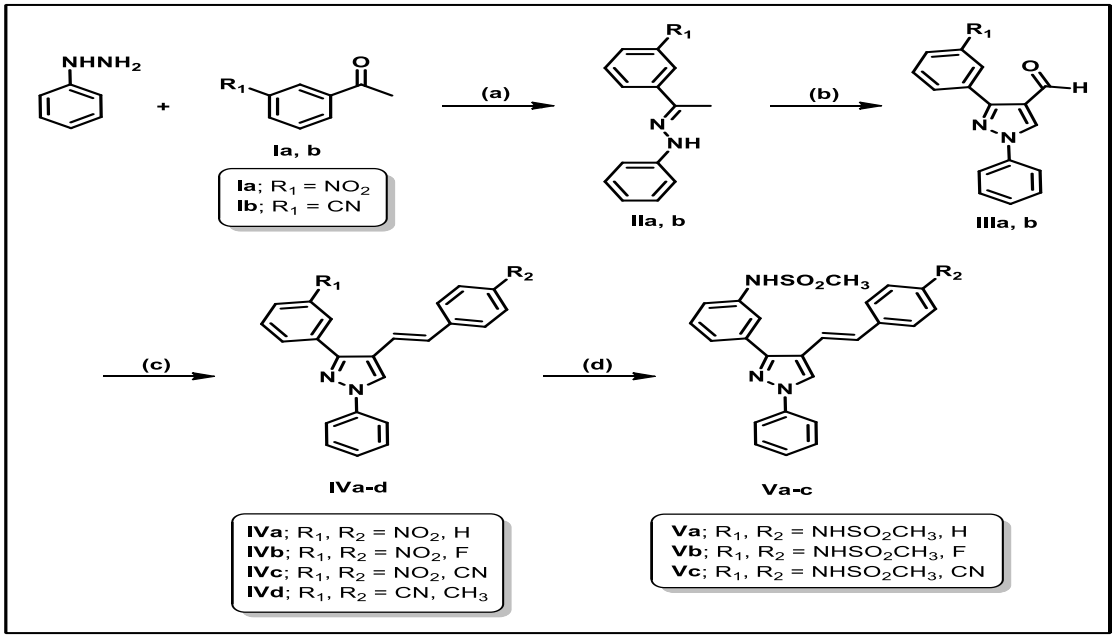
3.1. Chemistry

3.1.1. Synthesis of pyrazole derivatives

The synthesis of the desired pyrazole derivatives **VIc, d, Va-c** was achieved through the synthetic route outlined in **Scheme 1**. A mixture of concentrated nitric acid and concentrated sulfuric acid at specific proportions was added to a precooled solution of acetophenone at 0 °C to give the nitroaromatic intermediate **(Ia)** in 43% yield **[14].** Subsequently, phenylhydrazone intermediates **(IIa, b)** were attained in 76-77% yield from **(Ia, b)** after reaction with phenylhydrazine in acetic acid/ water mixture at room temperature **[15]**. Formylpyrazoles **(IIIa, b)** were obtained via Vilsmeier-Haack reaction [16] of the appropriate phenylhydrazones (**IIa, b)** in 87-88% yield. Compounds **(IVa-d)** were synthesized via Wittig reaction by reacting to the 4-substituted benzyltriphenylphosphonium bromide with NaH, then adding the formed ylide to the appropriate aldehyde **(IIIa, b)** afford the targeted compounds **(VIc, d)** and the alkene intermediates **(IVa, b)** in 78-80% yield. Compounds **(Va-c)** were prepared in two steps by reducing the nitro derivatives **(IVa, b, c)** using iron/NH_4Cl in ethanol/H_2O under refluxing conditions [17] followed by the nucleophilic substitution reaction of the amine with methanesulfonyl chloride (Hinsberg reaction **[18])** to generate the final compounds (**Va, b, c**) in 40-42% yield.



Scheme 1a, Reagent, and conditions: **(a)** H_2SO_4, HNO_3, 0 °C to rt, 15 min, 43%.

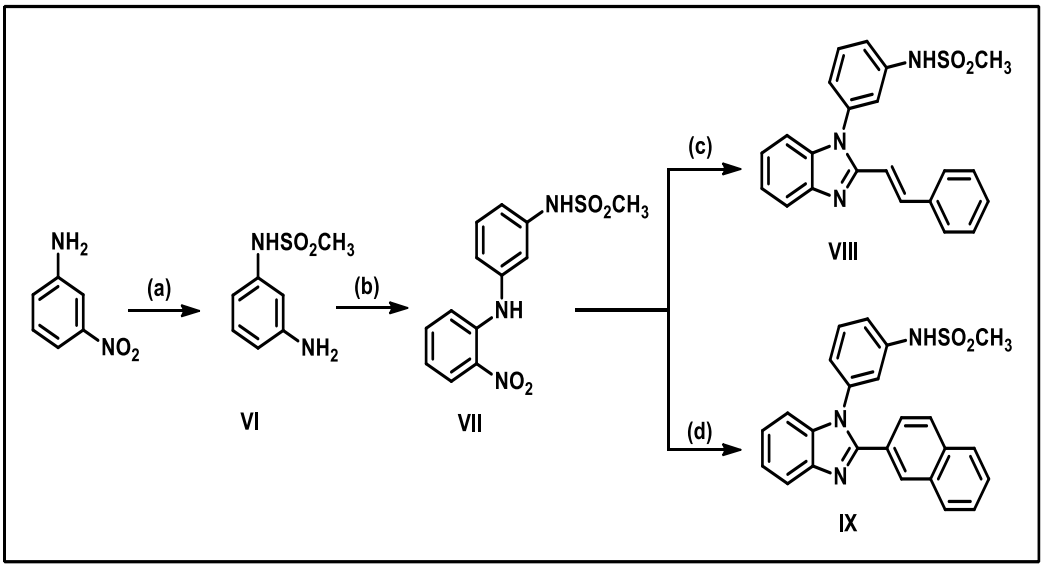


Scheme 1b, Reagent and conditions: (a) Glacial AcOH, H_2O, rt, overnight, 76-77%, (b) $POCl_3$, DMF, rt, overnight, 87-88%, (c) **[1]** Substituted benzyl halide, triphenylphosphine, anhydrous toluene, reflux, 6 hr, 87-90%, [2] IIIa, b derivatives, NaH, anhydrous DCM, under N_2, rt, overnight 78-80%, (d) **[1]** IVa, b, c derivatives, Fe, NH_4Cl, H_2O/ethanol, reflux, 6 hr, 39-45% , **[2]** $MeSO_2Cl$, pyridine, rt, overnight, 40-42%.

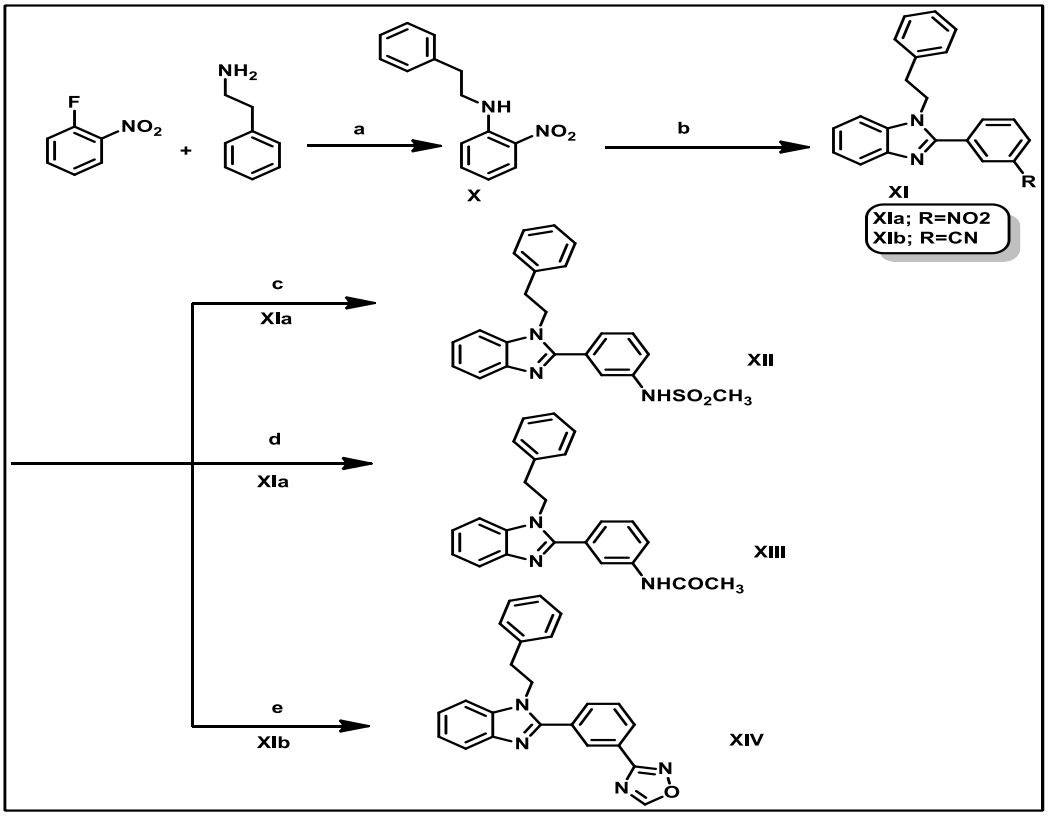
3.1.2. Synthesis of benzimidazole derivatives

The synthesis of the desired benzimidazole derivatives **VIII, IX, XII, XIII, XIV**was achieved through the synthetic route outlined in **Scheme 2**. Intermediate **(VI)** was obtained on two steps in 35.2% yield, by methylation of the amine group in pyridine **[19]**, then reduction of the nitro group with iron/NH_4Cl in ethanol/H_2O **[17]**. Then, 2-nitrofluorobenzene was reacted with the amine derivative **(VI)** using triethylamine and DMSO **[20]** to furnish the desired intermediate **(VII)** in 60% yield. The final benzimidazole compounds **(VIII, IX)** were synthesized via reductive cyclization of compound **(VII)** with the corresponding aldehyde in presence of DMSO as solvent and sodium dithionite as a reducing agent **[21]** to afford the final compounds **(VII, IX)** in 60-69% yield.

To prepare the final compounds **XII, XIII, XIV**, 2-nitrofluorobenzene was reacted with 2-phenylethanolamine using potassium carbonate in DMF to give intermediate **(X)** in 94% yield. This intermediate was subjected to reductive cyclization with the corresponding aldehyde in presence of DMSO as solvent and sodium dithionite as a reducing agent **[21]** to afford the desired benzimidazole intermediates **(XIa, b)** in 50-54% yield. Compound **(XIa)** was reduced using stannous chloride dihydrate in EtOAc to give the desired amino derivatives in good yields, then further methylation was done using mesyl chloride in pyridine to afford the final sulfacetamide derivatives (**XII**) in 50% yield. In addition, further acylation of the amine intermediate was achieved by using acetic anhydride in DCM to give the final acetamido derivatives (**XIII**) in 57% yield. Compound **(XIV)** was obtained on two steps in 37% yield, the oxime was synthesized first using hydroxylamine HCl and sodium carbonate in methanol under refluxing conditions overnight. Then, the residue was taken as such in excess triethyl orthoformate to form the 1,2,4-oxadiazole derivative.



Scheme 2a, Reagent and conditions: **(a) [1]** $MeSO_2Cl$, pyridine, rt, overnight, 76%, **[2]** Fe, NH_4Cl, H_2O/ethanol, reflux, 6 h, 35.2%, **(b)** 2-Fluoronitrobenzene, TEA, DMSO, 90 °C, overnight, 60%, **(c)** Cinnamaldehyde, Sodium dithionite, DMSO, 90 °C, overnight, 69%, **(d)** 2-Naphthaldehyde, Sodium dithionite, DMSO, 90 °C, overnight, 60%.



Scheme 2b, Reagent and conditions: **(a)** k_2CO_3, DMF, reflux, overnight, 94%, **(b)** aldehyde, Sodium dithionite, DMSO, 90 °C, overnight, 50-54%, **(c) [1] XIa,** $SnCl_2.2H_2O$, EtOAc, reflux, overnight, **[2]** $MeSO_2Cl$, pyridine, rt, overnight, 50%, **(d) [1] XIa,** $SnCl_2.2H_2O$, EtOAc, reflux, overnight, **[2]** Ac_2O, DCM, rt, overnight, 57%, **(e) [1] XIb,**, $NH_2OH.HCl$, Na_2CO_3, MeOH, reflux, overnight, **[2]** excess triethylorthoformate, reflux, overnight, 37%.

3.2. Biological evaluation

3.2.1. Initial screening of the synthesized pyrazoles and benzimidazoles against different *S. aureus* strains.

The minimum inhibitory concentrations (MICs) of the synthesized pyrazole derivatives, benzimidazole derivatives, and the control antibiotics (linezolid, vancomycin) were initially determined against MSSA (ATCC6538) and MRSA (USA300) strains (**Table 1**). Three compounds (**XII, XIII, XIV**) exhibited moderate antibacterial activity, inhibiting the growth of *S. aureus* and MRSA at concentrations ranging from 32 to 128 µg/mL. Based on the initial results obtained, further screening of these three active compounds (**XII**, **XIII**, **XIV**) was performed against additional clinical isolates of MSSA, MRSA, and VRSA, as presented in **(Table 2)**. Additionally, we examined whether the compounds were bacteriostatic or bactericidal by determining the minimum bactericidal concentration (MBC). Compound (**XII)** was found to be more active against VRSA (MIC of 16 or 32 µg/mL) compared to strains of MRSA and appears to be bactericidal (MIC matches or is less than four-fold the MBC values). Compounds (**XIII)** and (**XIV)** were generally less active than compound (**XII)** against both MSSA and MRSA

3.3. Molecular Modeling studies

3.3.1. Docking study

To correlate the antibacterial activity of our compounds against MRSA strains to the postulated target; PBP2a, we performed molecular docking via the C-docker protocol in Discovery Studio software. The docking was performed into PBP2a that is co-crystallized with the lead compound (*E*)-3-(3-carboxyphenyl)-2-(4-cyanostyryl) quinazoline-4(3*H*)-one (PDB code: 4CJN) **[11]**. The co-crystallized quinazolinone showed the essential interactions with the amino acids Lys-273, Lys-316, and Tyr-105 inside the PBP2a allosteric site as shown in **Fig. 3.** The three active compounds (**XII, XIII, XIV)** displayed very similar binding mode as the lead with comparable docking score.

Compound **XII** showed - C-docker energy of 28 Kcal/mol, which is comparable to the lead binding score of 32 Kcal/mol. Additionally, it retained the essential binding features of quinazolinone binding such as hydrogen bonding with Lys-273 and Lys 316 and pi-pi interactions with Lys-273 as shown in **Fig. 5**. Compound **XIII** docking score was 26 Kcal/mol and it displayed very similar binding mode to the lead compound and formed hydrogen bonding with Lys-273 and pi-pi interaction with Lys-273 but missed the hydrogen bonding with the key amino acid Lys-316 which might explain its lower activity compared to compound **XII** (**Fig.6)**. Moreover, compound **XIV** docking score was 25 Kcal/mol and displayed the same binding mode but it formed one pi-sigma interaction with Lys-316, while the other interactions were not observed **(Fig. 7)**. These findings might illustrate why compound **XIV** showed the least antibacterial activity within those three compounds.

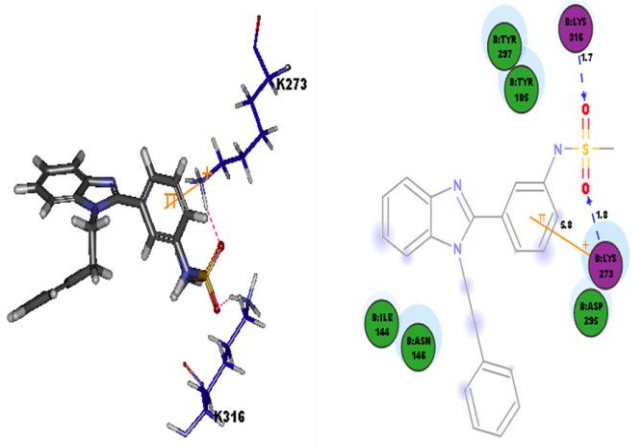
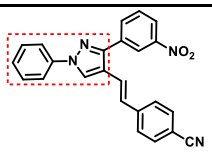
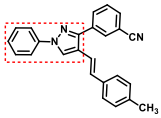
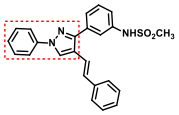
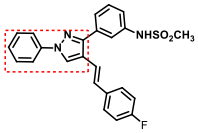
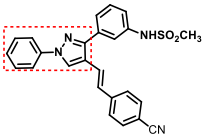
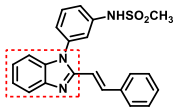
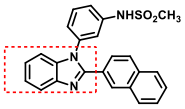
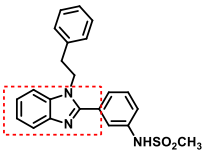
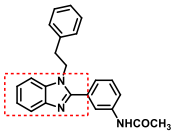
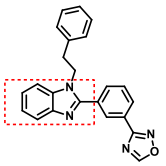


Fig. 5. 2D and 3D Binding pattern of the target compound **XII**

Table 1. MIC (µg/mL) of compounds and control antibiotics (linezolid, and vancomycin) screened against *Staphylococcus aureus* strains cultivated on tryptone soya broth (TSB) medium

Compound	Structure	MSSA ATCC 6538	MRSA NRS 384 (MRSA USA300)
IVc		> 32	> 32
IVd		> 128	> 128
Va		NT	>64
Vb		NT	>64
Vc		NT	>64
VIII		> 128	> 128
IX		> 128	> 128
XII		32	32
XIII		64	64
XIV		128	128
Linezolid		1	1
Vancomycin		1	1

NT, not tested

Table 2. MIC (µg/mL) and minimum bactericidal concentration (MBC in µg/mL) of the five active compounds against MRSA and VRSA and *Staphylococcus epidermidis* strains cultivated on tryptone soya broth (TSB) medium

Compound/ Control AB	Bacterial Strains															
	S. aureus ATCC 6538		*S. aureus* NRS 107		MRSA NRS 119		MRSA NRS 123 (USA400)		MRSA NRS 384 (USA300)		VRSA 10		VRSA 11a		VRSA 12	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
XII	32	64	64	> 64	> 64	> 64	16	64	64	> 64	16	64	16	16	32	32
XIII	64	> 64	> 64	> 64	> 64	> 64	64	> 64	> 64	> 64	64	> 64	64	> 64	64	> 64
XIV	> 64	>64	> 64	>64	> 64	> 64	64	> 64	> 64	>64	64	> 64	> 64	>64	> 64	>64
Mupirocin	≤0.5	≤0.5	> 64	> 64	≤0.5	≤ 0.5	≤0.5	1	≤0.5	≤0.5	≤0.5	≤0.5	0.25	16	≤0.5	≤0.5
Linezolid	1	2	1	2	32	64	1	2	2	2	1	16	1	4	1	4
Vancomycin	1	1	2	2	1	1	1	1	2	4	> 64	> 64	> 64	> 64	64	64

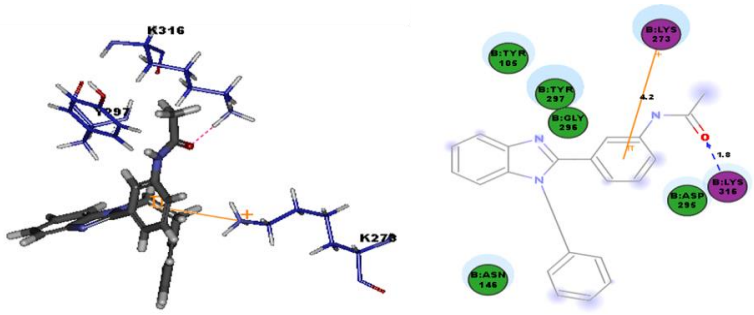


Fig. 6. 2D and 3D Binding pattern of the target compound **XIII**

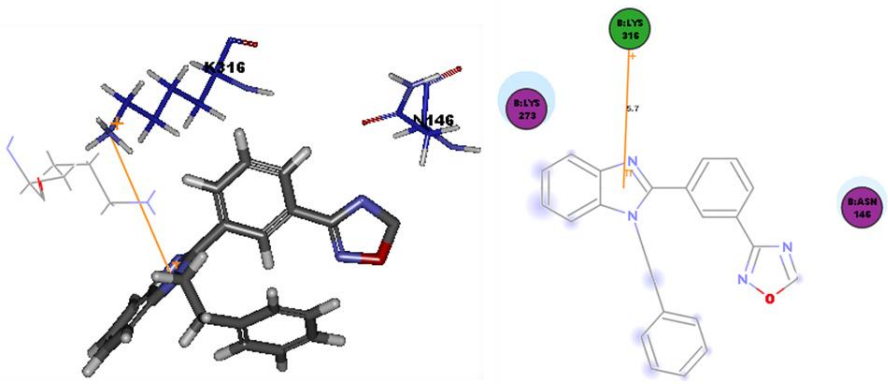


Fig. 7. 2D and 3D Binding pattern of the target compound **XIV**

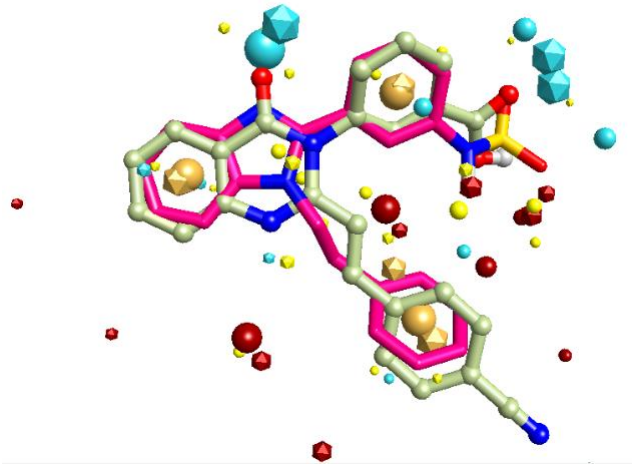


Fig. 8. Field alignment of compound **8** and quinazolinone based antibiotic showing similar molecular fields suggesting a similar binding mode to PBP2a. Spherical field points: compound **XII**, icosahedral field points: reference compound. Cyan: Negative field points, Red: Positive field points, Yellow: van der Waals surface field points, Gold: Hydrophobic field points, compound **XII** is displayed in magenta

3.3.2. Field alignment study

Field alignment of the most potent compound (**XII)** was performed against the bioactive conformation of the lead compound (PDB code: 4CJN) **[11]** as a reference using Cresset's FieldAlign® module, version 2.0.1, which showed that the designed compound has the same electronic fields as the lead compound, meaning that the interactions of the compound with the protein is similar to that of the lead (**Fig. 8**). Besides, the alignment agrees with our predicted binding mode from the docking study. It should be mentioned that compound (**XII)** which had the highest inhibitory potency (MIC = 16 µg/mL) showed the best alignment with the highest score; 0.725

Conclusion

The antibiotic resistance of MRSA is attributed to the high molecular mass transpeptidase enzyme, the penicillin-binding protein 2a (PBP2a). The commercially available β-lactams failed to inhibit this resistant enzyme and only a few compounds were reported to have a high affinity to it. Unfortunately, the resistance to these antibiotics has been recorded in the last few decades which imposes a huge need to develop non-β lactam small molecules to inhibit PBP2a. Our goal is to develop novel potent small molecules effective against MRSA pathogen to offer a long-sought treatment option for life-threatening infections not responding to conventional antibiotics. The rational of our design was scaffold hopping of the lead compound (*E*)-3-(3-carboxyphenyl)-2- (4-cyanostyryl)quinazoline-4(3*H*)-one, we replaced the quinazolinone core with phenyl pyrazole and benzimidazole scaffolds. Preliminary modeling studies were carried out to ensure the compliance of the design strategy with the reported binding mode of the lead compound. Subsequently, the synthesized compounds were selected to be tested for their antimicrobial activity against MRSA strains. Fortunately, our study has born a moderately active compound (**XII)** that showed a considerable MIC of 16 µg/mL rendering it a good start for optimization to introduce a new class of PBP2a inhibitors as potential anti-MRSA agents.

5. Experimental

5.1. Chemistry

Chemicals were purchased from Sigma-Aldrich (Germany), Merck (Darmstadt, Germany), Alfa Aesar (Germany) and Loba Chemie (India), and were used as such without further purification. Solvents used for column chromatography were redistilled before use on BUCHI Rotavapor. Flash column chromatography was performed using silica gel (230-400 mesh particle size) purchased from Sigma-Aldrich. Reactions were followed using analytical thin-layer chromatography (TLC), performed on Aluminum silica gel 60 F254 TLC plates, purchased from Merck, with visualization under UV light (254 nm). Melting points were recorded on the Stuart Scientific apparatus and are reported herein uncorrected. Routine ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on Bruker 400 MHz spectrometer in δ scale (ppm), using DMSO as solvent and TMS as the internal standard signal at the Center for Drug Discovery Research and Development, faculty of pharmacy, Ain Shams University.

5.1.1. General synthetic procedures for compounds VIc, d; Va,b,c

Intermediate (Ia) was prepared following similar previously reported procedures **[22]**.

Step a: To a solution of the appropriate acetophenone derivative (**Ia, b**) (1.0 equiv., 24.2 mmol) in acetic acid (50 mL) and water (5 mL), phenylhydrazine (1.0 equiv., 24.2 mmol, 2.6 g, 2.4 mL) was added. The reaction mixture was allowed to stir overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into cold water (50 mL), filtered, and dried to yield the designated compounds (**IIa, b**) in 76-77%.

Step b: DMF (9.2 equiv., 435 mmol, 31.81 g, 33.7 mL) was placed in a 100 mL flask and cooled in an ice/salt bath to 0 °C. Phosphorus oxychloride (2.2 equiv., 47.2 mmol, 7.2 g, 4.5 mL) was added dropwise with stirring until a yellow solution was formed. The appropriate hydrazine derivative (**IIa, b**) (1.0 equiv., 21 mmol) was added portion-wise to the reaction mixture and stirred overnight at room temperature. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (250 mL), neutralized to pH 6-8 using cold saturated K_2CO_3 solution, filtered, washed with water (3 X 100 mL), and dried to yield the designated compounds (**IIIa, b**) in 87-88% yield.

Step c: To a solution of the appropriate benzyl bromide (1.0 equiv., 5.1 mmol) in toluene (20 mL), triphenylphosphine (1.0 equiv., 5.1 mmol, 1.54 g) was added. The mixture was then heated under reflux for 6 h, where a heavy precipitate was formed. Upon completion of the reaction, the reaction mixture was cooled to room temperature and the precipitate was filtered, triturated with toluene to yield the desired 4-substituted benzyltriphenylphosphonium bromide derivatives as a white solid in excellent yields (87-90%) that were used as such in the next step.

To a well stirred suspension of the appropriate 4-substituted benzyltriphenyl-phosphonium bromide (1.0 equiv., 1.7 mmol) and the appropriate aldehyde (**IIIa, b**) (1.0 equiv., 1.7 mmol) in DCM (20 mL) under a nitrogen at 0 °C, sodium hydride (3 equiv., 5.1 mmol, 0.122 g) was added portion wise. The reaction mixture was stirred at room temperature for 24 h. Upon completion of the reaction as indicated by TLC, the excess sodium hydride was quenched by the addition of cold water (50 mL). The aqueous phase was extracted by DCM (3 X 50 mL) and the organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography to give the desired compounds **(IVa,b)** and the final compounds

(IVc,d) in 78-80%.

(*E*)-3-(3-Nitrophenyl)-1-phenyl-4-styryl-1*H*-pyrazole (IVa). R_f= 0.25 (DCM/MeOH 9:1). Greenish yellow solid (0.38 g, 80%); MP 150-152 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.51 (s, 1H), 8.30 (d, J= 8.1 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 7.98 (d, J= 8.0 Hz, 2H), 7.83 (t, J = 8.0 Hz, 1H), 7.62 – 7.53 (m, 4H), 7.45 – 7.32 (m, 3H), 7.29 (t, J= 7.7 Hz, 1H), 7.24 – 7.14 (m, 2H).

(*E*)-4-(4-Fluorostyryl)-3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole (IVb). R_f= 0.25 (DCM/MeOH 9:1). Greenish yellow solid (0.39 g, 78%); MP 130-132 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 8.59 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 2H), 7.96 (s, 1H), 7.83 – 7.71 (m, 3H), 7.70 – 7.62 (m, 3H), 7.62 – 7.54 (m, 1H), 7.48 (d, *J*= 8.0 Hz, 1H), 7.33 (t, *J*= 8.8 Hz, 2H), 6.90 (d, *J*= 12.0 Hz, 1H), 6.79 (d, *J*= 12.0 Hz, 1H).

(*E*)-4-(2-(3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)vinyl)benzonitrile (IVc). R_f= 0.25 (DCM/MeOH 9:1). White solid (0.4 g, 80%); MP 160-162°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.13 (s, 1H), 8.50 (s, 1H), 8.31 (d, *J*= 8.4 Hz, 1H), 8.21 (d, *J*= 7.7 Hz, 1H), 7.97 (d, *J*= 8.0 Hz, 2H), 7.89–7.78 (m, 3H), 7.74 (d, *J*= 8.1 Hz, 2H), 7.58 (t, *J*= 7.8 Hz, 2H), 7.46–7.34 (m, 2H), 7.25 (d, *J*= 16.2 Hz, 1H).

(*E*)-3-(4-(4-Methylstyryl)-1-phenyl-1*H*-pyrazol-3-yl)benzonitrile (IVd). R_f= 0.25 (DCM/MeOH 9:1). Bright yellow Solid (0.37 g, 80%); MP 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.02 (s, 1H), 8.15 (t, *J*= 1.7 Hz, 1H), 8.11–8.03 (m, 1H), 8.01–7.88 (m, 3H), 7.75 (t, *J*= 7.9 Hz, 1H), 7.56 (t, *J*= 7.9 Hz, 2H), 7.48–7.33 (m, 3H), 7.19 (d, *J*= 7.9 Hz, 2H), 7.10 (d, *J*= 4.7 Hz, 2H), 2.31 (s, 3H).

Step d: To a solution of the appropriate nitro derivative **(IVa,b,c)** (1.0 equiv., 2.5 mmol) in ethanol/water (3:1) (50 mL), iron powder (3.0 equiv.,8.2 mmol, 0.45 g) and ammonium chloride (9.0 equiv., 24.3 mmol, 2.3 g) were added. The reaction mixture was heated under reflux for 6 hr. Upon completion of the reaction as indicated by TLC, the reaction mixture was filtered on celite and the filtrate was concentrated under reduced pressure. The resulting residue was extracted with EtOAc (3 X 100 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, concentrated, and triturated with diethyl ether to give the corresponding aniline derivatives as orange powder in moderate yields (39-45%) that was used as such in the next step.

A solution of the appropriate aniline derivative (1.0 equiv., 1 mmol) in pyridine (5 mL) was cooled in an ice bath and methanesulfonyl chloride (1.2 equiv., 1.2 mmol, 0.137 g, 92.8 mL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into acidified ice/water (50 mL), then extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, evaporated and the resulting residue was purified by flash chromatography to give the designated compounds **(Va-c)** in 40-42%.

(*E*)-*N*-(3-(1-Phenyl-4-styryl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide (Va). R_f= 0.30 (EtOAc/Hexane 3:1). Buff solid (0.17 g, 42%); MP 138-140 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.93 (s, 1H), 9.03 (s, 1H), 7.95 (d, *J*= 8.0 Hz, 2H), 7.59–7.49 (m, 6H), 7.42–7.24 (m, 6H), 7.16 (s, 2H), 3.05 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 40.2, 118.47, 118.81, 119.82, 119.87, 120.26, 123.98, 126.57, 126.63, 127.04, 127.86, 128.81, 129.22, 129.63, 130.07, 130.28, 134.25, 137.65, 139.23, 139.73, 150.45.

(*E*)-*N*-(3-(4-(4-Fluorostyryl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide (Vb). R_f= 0.30 (EtOAc/Hexane/MeOH 4:4:0.25). Yellow solid (0.18 g, 41%); MP 109-111°C; ^{1}H

NMR (400 MHz, DMSO-d6) δ 9.84 (s, 1H), 8.32 (s, 1H), 7.82 (d, *J*= 8.1 Hz, 2H), 7.69 (s, 1H), 7.56–7.44 (m, 3H), 7.44–7.35 (m, 3H), 7.35–7.27 (m, 1H), 7.22 (d, *J*= 8.0 Hz, 1H), 7.09 (t, *J*= 8.8 Hz, 2H), 6.68 (d, *J*= 12.0 Hz, 1H), 6.54 (d, *J*= 12.0 Hz, 1H), 3.00 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 40.21, 115.56, 115.77, 117.63, 118.79, 118.96, 119.67, 120.53, 123.20, 126.98, 127.76, 129.93, 130.84, 133.61, 134.31, 139.12, 139.63, 150.36, 160.41, 162.84.

(*E*)-*N*-(3-(4-(4-Cyanostyryl)-1-phenyl-1*H*-pyrazol-3-yl)phenyl)methanesulfonamide (Vc). R_f= 0.25 (EtOAc/Hexane/MeOH 4:2:0.25).Yellow solid (0.2 g, 41%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18–9.63 (m, 1H), 9.08 (s, 1H), 7.41 (s, 16H), 3.06 (s, 3H).

5.1.2. General synthetic procedures for compounds VIII-IX

Step a: A solution of 3-nitroaniline (1.0 equiv., 21.7 mmol, 3 g) in pyridine (20 mL) was cooled in an ice bath and methanesulfonyl chloride (1.2 equiv., 26.06 mmol, 2.97 g, 2 mL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into acidified ice/water (250 mL), stirred for 1 h, filtered, washed with water, and dried to yield *N*-(3-Nitrophenyl)methanesulfonamide as buff solid in 76.9% yield.

To a solution of *N*-(3-nitrophenyl)methanesulfonamide (1.0 equiv., 10 mmol, 2 g) in ethanol/water (3:1) (100 mL),), iron powder (3.0 equiv., 32.8 mmol, 1.8 g) and ammonium chloride (9.0 equiv., 97.6 mmol, 5.2 g) were added. The reaction mixture was heated under reflux for 6 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was filtered on celite and the filtrate was concentrated under reduced pressure. The resulting residue was extracted with EtOAc (3 X 100 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give the desired compound **(VI)** in 35.2% yield.

Step b: To a solution of *N*-(3-aminophenyl)methanesulfonamide **(VI)** (3.0 equiv., 3 mmol, 0.56 g) in DMSO (0.75 mL), 2-Nitroflurobenzene (1.0 equiv., 1 mmol, 0.14 g) and triethylamine (0.3 g) were added. The reaction mixture was heated at 90 °C overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (100 mL) with vigorous stirring, filtered, washed with water, and dried to yield the designated compound **(VII)** in 60% yield.

Step c: To a solution of *N*-(3-((2-nitrophenyl)amino)phenyl)methanesulfonamide **(VII)** (1.0 equiv., 0.65 mmol, 0.2 g) in DMSO (2 mL), cinnamaldehyde (1.1 equiv., 0.715 mmol, 0.094 g, 0.089 mL) and sodium dithionite (3.0 equiv., 1.95 mmol, 0.33 g) were added. The reaction mixture was heated at 90 °C overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water/brine (100 mL), filtered, washed with water and dried and the resulting residue was purified by flash chromatography to give the desired compound **(VIII)**.

(*E*)-*N*-(3-(2-Styryl-1*H*-benzo[*d*]imidazol-1-yl)phenyl)methanesulfonamide (VIII). R_f= 0.4 (EtOAc/Hexane1:6). Yellow solid (0.15 g, 60%); MP 118-120 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 10.15 (s, 1H), 7.90 (d, *J*= 15.9 Hz, 1H), 7.74 (d, *J*= 7.8 Hz, 1H), 7.66 (t, *J*= 8.0 Hz, 1H), 7.61 (d, *J*= 7.4 Hz, 2H), 7.45–7.31 (m, 7H), 7.31–7.23 (m, 2H), 7.00 (d, *J*= 15.9 Hz, 1H), 3.13 (s, 3H).

Step d: compound (**IX**) was prepared similarly to (**VIII**) using naphthaldehyde. The resulting residue was purified by flash chromatography.

***N*-(3-(2-(Naphthalen-2-yl)-1*H*benzo[d]imidazol-1yl)phenyl)methanesulfonamide (IX).** R_f= 0.2 (EtOAc/Hexane1:3). Yellow solid (0.18 g, 69%); MP 125-127°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 10.03 (s, 1H), 8.15 (s, 1H), 7.92 (dd, *J*= 8.2, 5.4 Hz, 2H), 7.89–7.81 (m, 2H), 7.66–7.49 (m, 4H), 7.41–7.30 (m, 4H), 7.27 (t, *J*= 2.1 Hz, 1H), 7.20 (d, *J*= 7.3 Hz, 1H), 2.86 (s, 3H).

5.1.2. General synthetic procedures for compounds XII, XIII, XIV

Step a: To a stirred solution of 2-nitroflurobenzene (1.0 equiv., 3.5 mmol, 0.5 g) in DMF (5 mL) and K_2CO_3 (5.0 equiv., 17.7 mmol, 2.4 g), 2-phenylethanamine (1.1 equiv., 3.8 mmol, 0.47 g) was added. The reaction mixture was heated at 90 °C overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water (100 mL) with vigorous stirring, filtered, washed with water, and dried to yield the designated compound **(X)** as bright orange solid in 94% yield.

Step b: To a solution of 2-nitro-*N*-phenylethylamine **(X)** (1.0 equiv., 2.06 mmol, 0.43 g) in DMSO (2 mL), corresponding aldehyde (3-nitrobenzaldehyde or 3-cyanobenzaldehyde (1.1 equiv., 2.26 mmol, 0.311 g) and sodium dithionite (3.0 equiv., 5.3 mmol, 0.92 g) were added. The reaction mixture was heated at 90 °C overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice/water/brine (100 mL), filtered, washed with water and dried to yield the designated compound **(XIa,b)** in 50-54% yield.

Step c: To a solution of the appropriate nitro derivative (1.0 equiv., 1.0 mmol, 0.35 g) in EtOAc (25 mL) was added $SnCl_2.2H_2O$ (5.0 equiv., 5.0 mmol, 1.1 g) and the reaction mixture was refluxed overnight. Upon completion of the reaction as indicated by TLC, the mixture was cooled, washed with sodium carbonate solution (10%), separated, dried. The amine intermediate was entered directly to the next step without further purification. A solution of amine intermediate (1.0 equiv., 1.0 mmol, 0.32 g) in pyridine (5 mL) was cooled in an ice bath and methane sulfonyl chloride (1.2 equiv., 1.2 mmol, 0.14 g, 0.09 mL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into acidified ice/water (100 mL), stirred for 1 h, filtered, washed with water, and dried and the resulting product was purified by flash chromatography to give the desired compound **(XII)** in 50% yield.

***N*-(3-(1-phenethyl-1*H*-benzo*[d]*imidazol-2-yl)phenyl)methanesulfonamide (XII).** R_f= 0.2 (DCM/MeOH 9.5:0.5). Off-white solid (0.19 g, 50%); MP 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.97 (s, 1H), 7.69 (dd, *J*= 7.6, 1.5 Hz, 2H), 7.53–7.44 (m, 2H), 7.44–7.13 (m, 7H), 6.98 (dd, *J*= 6.8, 2.7 Hz, 2H), 4.52 (t, *J*= 7.4 Hz, 2H), 3.08–2.96 (m, 5H).

Step d: To a solution of the appropriate nitro derivative (1.0 equiv., 1.0 mmol, 0.35 g) in EtOAc (25 mL) was added $SnCl_2.2H_2O$ (5.0 equiv., 5.0 mmol, 1.1 g) and the reaction mixture was refluxed overnight. Upon completion of the reaction as indicated by TLC, the mixture was cooled, washed with sodium carbonate solution (10%), separated, dried. The amine intermediate was entered directly to the next step without further purification. A solution of amine intermediate (1.0 equiv., 1.0 mmol, 0.32 g) in DCM (20 ml) was added acetic anhydride (1.5 equiv., 1.5 mmol, 0.1 g, 94 µL) and the reaction mixture was stirred at room temperature overnight. Upon completion of the reaction as indicated by TLC, the mixture was evaporated, purified by column chromatography to yield the desired final products (**XIII**).

***N*-(3-(1-phenethyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl)acetamide (XIII).** R_f= 0.2 (DCM/MeOH 9.5:0.5). White solid (0.16 g, 57%); MP 185-188 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 10.15 (s, 1H), 8.00 (d, *J*= 2.0 Hz, 1H), 7.72–7.64 (m, 3H), 7.45 (t, *J*= 7.9 Hz, 1H), 7.34–7.11 (m, 6H), 7.01 (dd, *J*= 7.1, 2.4 Hz, 2H), 4.50 (t, *J*= 7.6 Hz, 2H), 3.01 (t, *J*= 7.5 Hz, 2H), 2.10 (s, 3H).

Step e: To solution of intermediate **(XIb)** (1.0 equiv., 6.1 mmol, 1.9 g) in methanol were added hydroxylamine.HCl ($NH_2OH.Cl$) (2.0 equiv., 12.2 mmol, 0.85 g) and Na_2CO_3 (2.0 equiv., 12.2 mmol, 1.3 g) and the reaction mixture was refluxed overnight. Upon completion of the reaction as indicated by TLC, the mixture was cooled, evaporated under vaccum and used directly in the final step without further purification. The residue was dissolved into excess triethylorthoformate under reflux conditions overnight. Upon completion of the reaction as indicated by TLC, the mixture was evaporated, purified by column chromatography to yield the desired final products (**XIV**).

3-(3-(1-phenethyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1,2,4-oxadiazole (XIV). R_f= 0.2 (DCM/MeOH 9.5:0.5). Beige solid (0.8 g, 37%); MP 200-204 °C ^{1}H NMR (400 MHz, DMSO-d6) δ 8.24–8.12 (m, 2H), 8.04 (d, *J*= 7.7 Hz, 1H), 7.78–7.66 (m, 3H), 7.59 (t, *J*= 7.7 Hz, 1H), 7.37–7.25 (m, 2H), 7.19–7.08 (m, 3H), 6.95 (dd, *J*= 6.4, 2.9 Hz, 2H), 4.52 (t, *J*= 7.4 Hz, 2H), 3.01 (t, *J*= 7.4 Hz, 2H).

5.2. Biological Assays

5.2.1. Determination of MIC and MBC against different strains of pathogenic Staphylococci.

The MICs of the tested compounds and control antibiotics (linezolid, and vancomycin) were initially determined using the broth microdilution method, according to guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) **[23]**, against MSSA (ATCC6538), and MRSA (USA300) using TSB. Two-fold serial dilutions were done in a 96 well-plate (testing concentrations from 128 down to 1 µg/mL initially) and then incubated at 37 ºC for 20-24 hours before determining the MIC. All the compounds were prepared in 10 mg/mL stock solutions except for compound **IVc** (prepared to 1 mg/mL stock in DMSO). The MBC of the active compounds was determined by plating 5 µL from each well where no growth was present (in the MIC plates) onto tryptic soy agar (TSA) plates and incubating plates at 37 ºC for 24 h. The MBC was categorized as the lowest concentration that generated a 99.9% reduction in bacterial colony-forming units.

5.3. Molecular modeling

5.3.1. Molecular docking

A molecular docking study was performed using the C-Docker 2.5 module of Accelrys discovery studio 2.5 (Accelrys Inc., San Diego, CA, USA) at the Faculty of Pharmacy, Ain Shams university, drug design laboratory.

5.3.1.1. Preparation of protein

The X-ray crystal structure of PBP2a co-crystallized with (*E*)-3-(3-carboxyphenyl)-2-(4-cyanostyryl)quinazoline-4(3*H*)-one was downloaded from the Protein Data Bank at the Research Collaboration for Structural Bioinformatics (RCSB) website [www.rcsb.org] (PDB code: 4CJN) and loaded in Accelrys discovery studio 2.5. The preparation of the protein structure was performed using the default protein preparation tools built into the software. Adding the missing hydrogen atoms to the amino acid residues was the first step in the preparation process. Then, completing the missing residues and applying force field parameters were done using CHARMm forcefield. Due to the addition of hydrogens, clashes occur, so the whole structure of the protein was minimized using a

minimization protocol. Fixed constraints have been created on the heavy atoms other than hydrogen during the minimization process. All the protein was defined as the receptor. The binding pocket and the surrounding amino acid residues were collectively defined as binding site sphere. Before running the docking process the ligand was deleted from the binding site.

5.3.1.2. Ligand preparation prior docking process

The structure of the ligands was drawn using the default sketching tools of Accelrys discovery studio 2.5. The preparation of the ligands was conducted using the Ligand preparation protocol of Accelrys Discovery Studio. The ionization pH parameter was adjusted to 7.4 and hydrogen atoms were added. The parameter of isomer and tautomer generation was unchecked.

5.3.1.3. Docking process

CDOCKER protocol was used to dock our compounds into the allosteric site of PBP2a. After running the protocol, ten docking poses were generated for each ligand docked and were thoroughly inspected for getting the best binding mode. The top-ranked poses were selected and investigated. The docking scores are displayed in energy terms (CDOCKER Energy). The higher the score (in negative terms), the better the binding affinity.

5.3.2. Field alignment study

The study was carried out using Cresset FieldAlign software where the reference molecule was supplied in the bioactive conformer obtained from the PDB structure (PDB code: 4CJN). Database molecules were imported from the saved pdf file. Before the alignment to the reference molecule and the addition of molecular field point, a conformation generation protocol was applied within FieldAlign to each molecule in the database. The results of the alignment process were ranked in descending order and manually we could choose the best conformer.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

The authors have declared no conflict of interest.

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Authors' contributions

The manuscript was drafted and written by Menna-Allah W. Shalaby. Eman M. E. Dokla, Rabah A. Taha and Khaled A. M. Abouzid has provided comments and contributed to revising the manuscript. All authors have read and approved the final manuscript.

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