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Original article

Synthesis and in vitro anti-tumor activity of new oxadiazole thioglycosides

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ABSTRACT

A facile, convenient and high yielding synthesis of novel thioglycosides incorporating 1,3,4-oxadiazole, triazole and or triazine moieties from readily available starting materials has been described. The key step of this protocol is the formation of 3-*iso*butyl-1-phenyl-*1H*-pyrazole-4-carbaldehyde (**3**) *via* condensation between methyl *iso*-butyl ketone and phenylhydrazine followed by application of Vilsmeier–Haack reaction. **3** was converted either to 1,3,4-oxadiazole derivative or condensed with *O*-aminothiols to give the bases **8**, **19** and **20** in good yields, respectively. The aglycons **8**, **19**, and **20** were coupled with different activated halosugars in the presence of basic medium. Pharmacological evaluation of compounds **8**, **14**, **16** and **22** in vitro against 2-cell lines MCF-7 (breast) and HEPG2 (liver) revealed them to possess high anti-tumor activities with IC_{50} values ranging from 2.67–20.25 (μg/mL) for breast cell line (MCF-7) and 4.62–43.6 (μg/mL) for liver cell line (HEPG2). None of the tested compounds exhibited any toxicity in doses up to 500 mg kg^{-1} of the animal body weight.

1. Introduction

The incidence and mortality of cancer patients have become one of the important issues discussed worldwide. Unfortunately, development of resistance to chemotherapeutic agents is a common obstacle in the treatment of different types of cancers [1,2]. Several important drugs including tamoxifen (TAM), 5-flurouracil (5FU), adriamycin (ADR) and vincristin (VCR) with different structures and mechanisms of anti-tumor activities fail to end these problems completely. Due to the several side effects, drug resistance and failure of anti-tumor drugs to exert their effects in certain cases of cancers [3–5], looking for new chemotherapeutic agents with synthetic or natural origins is one of the hot topics in cancer research laboratories.

1,3,4-Oxadiazole derivatives have attracted significant attention in the field of drug discovery because of their wide array of pharmacological activities, including antibacterial–antifungal, analgesic, anti-inflammatory, antihypertension, muscle relaxing and anticancer activities [6–16]. Additionally, pyrazole and N-arylpyrazole derivatives are very important class of heterocyclic compounds that have remarkable pharmacological activities as antibacterial–antifungal, hypoglycemic, hyperlipidemia, kinase inhibitor for treatment of type 2 diabetes, anti-inflammatory and tumor necrosis inhibitor [17–21].

In view of the above mentioned findings and our previous reports [22–26], the purpose of the present work was to design, synthesize and investigate the anti-tumor activity of some novel 1H-pyrazolo-1,3,4-oxadiazole derivatives carrying carbohydrate residues through S-glycosidic bond formation.

2. Chemistry

The synthesis of our desired pyrazole derivatives began with commercially available aliphatic ketones **1a**,**b** (namely, 2-butanone and methyl *iso*-butyl ketone) and phenylhydrazine as starting materials. Condensation between the ketone and hydrazine provided a hydrazones **2a**,**b** in high yields. The Vilsmeier–Haack reaction using 2.5 eq of reagent performed a double addition of reagent to afford, ultimately after hydrolysis, the desired cyclized aldehyde **3** in 80% yield unfortunately, the application of the Vilsmeier–Haack reaction at the hydrazone **2a** did not led to desired product, instead 3,4-dimethyl-1-phenyl pyrazole (**4**) was obtained as a sole product (Scheme 1).

The reaction sequence of preparing the desired aglycon [5-(3-*Iso*butyl-1-phenyl-1H-pyrazole-4-yl)-1,3,4- oxadiazle-2-thiol (**8**)] is some what long and linear with few common intermediates. To this aim, aldehyde **3** is oxidized using a mixture of sulphuric acid (30%) and $K_2Cr_2O_7$ to yield the acid **5** which esterified using absolute ethanol in presence of few drops of conc. sulphuric acid, affording **6** in fairly good yield. The ^1H NMR of acid **5** showed the

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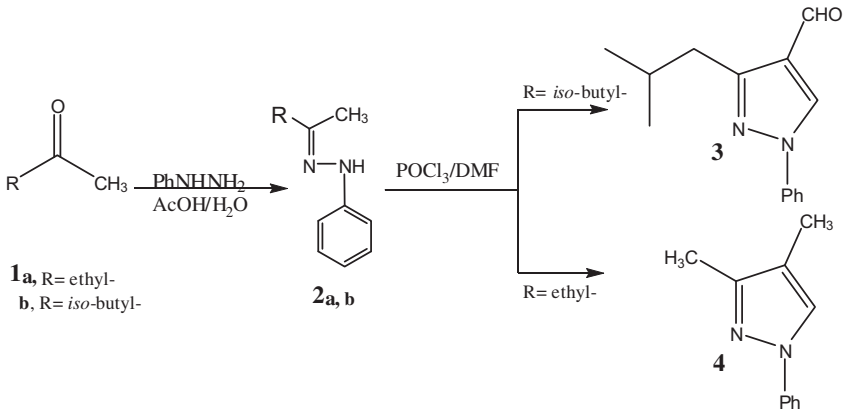


Scheme 1. Synthesis of 1-phenylpyrazoles **3** and **4**.

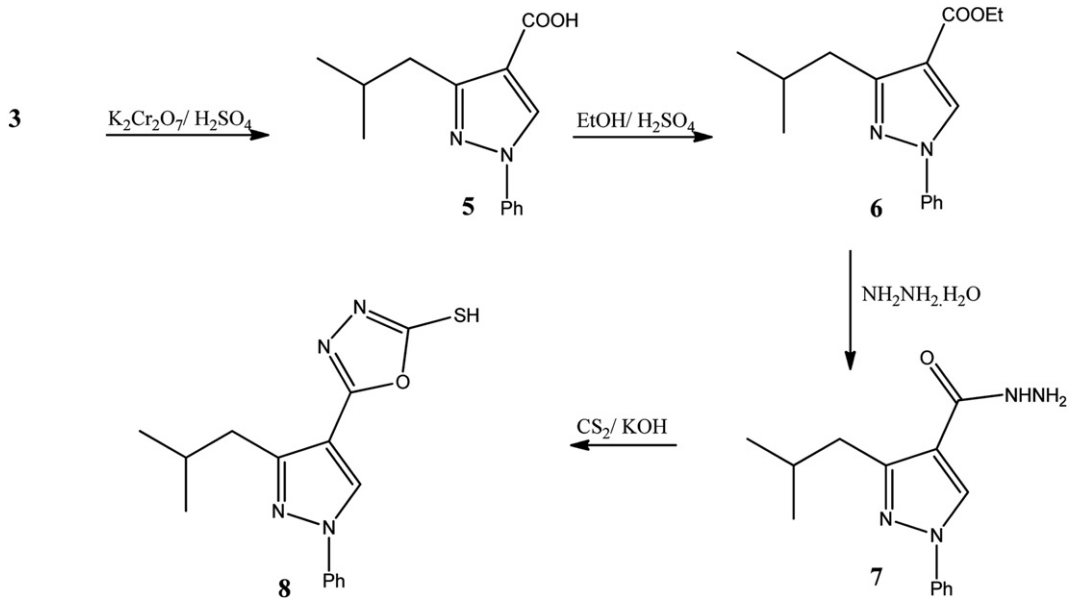
absence of aldehydic proton which detected at the parent **3**, while, the ester **6** showed the ethyl protons at their expected locations.

Treatment of **6** with hydrazine hydrate in boiling ethanol afforded the hydrazide derivative **7** in good yield. Finally the oxadiazole derivative **8** was achieved following our previously reported method [27] by adopting a simple one pot procedure that involves reacting of **7** with carbon disulphide under strong basic conditions followed by acidification with diluted hydrochloric acid (Scheme 2).

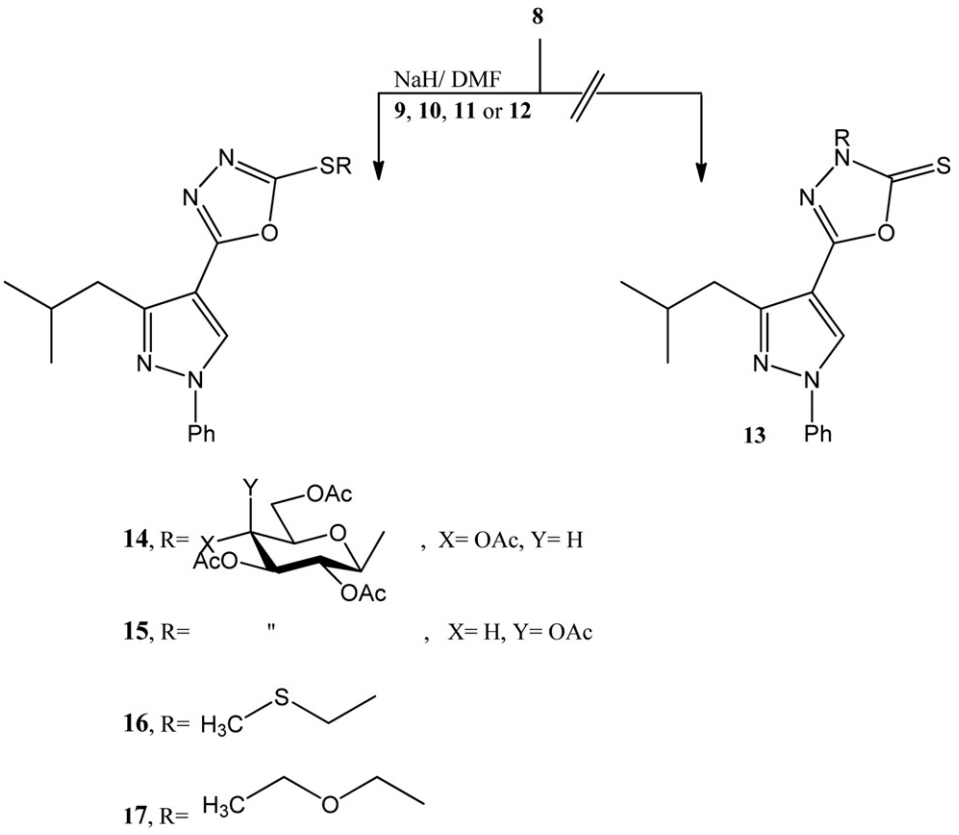
The coupling between the aglycon and the activated sugars was achieved in the presence of basic medium. Thus, aglycon **8** was reacted with NaH in dry DMF followed by addition of the activated cyclic sugars (2′,3′,4′,6′-tetra-*O*-acetyl-α-D-gluco (or galacto)) pyranosyl bromide **9** and **10**, or the activated acyclic sugar analogues, chloromethyl methylthioether (**11**), or chloromethyl ethyl ether (**12**), to give the corresponding thioglycoside derivatives **14**–**17** in a good yields, respectively.

The structures of thioglycosides **14** and **15** were established and confirmed for the reaction products on the basis of their elemental analysis and spectral data (^{1}H NMR, ^{13}C NMR and MS, cf. experimental). Thus, their ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ = 5.68 and 6.24 ppm with a spin–spin coupling constant ($J_{1',2'}$ = 9.95 and 10.39 Hz) corresponding to a *trans* orientation of H-1′ and H-2′ protons indicating the β-configuration. On the other hand, the formation of S-glycosides **14** and **15** and not the corresponding N-glycosides **13** were proved using ^{13}C NMR spectroscopy which revealed the absence of a signal at δ 178 ppm for the thione carbon and the appearance of a signal at δ = 162.21 and 161.55 ppm corresponding to the C-2 carbon of the 1,3,4-oxadiazole ring, whose chemical shift is the same as that of the corresponding S-methyl derivative [28–30], the same results were obtained with compounds **16** and **17** (Scheme 3).

Additionally, when compound **3** was condensed with selected examples of bidentate ligands (**18a**, **b**), the condensing adducts **19**, **20** were obtained in high yields. The ^{1}H NMR of **19**, **20** showed the methine CH proton at 7.97 and 8.22 ppm supported the condensing adduct structures. Finally, the S-glycosides **21**, **22** were obtained in fairly good yields, on treatment of compounds **19** and **20** with **9** in the presence of NaH/DMF following the above mentioned conditions whereas the β-isomers were obtained ($J_{1',2'}$ = 9.75 and 9.20 Hz) (Scheme 4).



Scheme 2. Synthetic pathway for the synthesis of 5-(3-isobutyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole-2-thiol **8**.

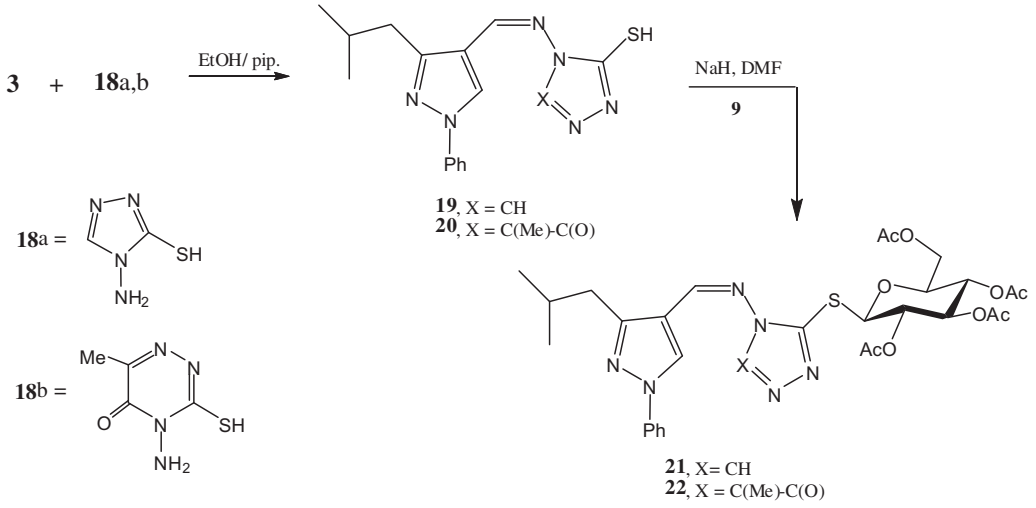


Scheme 3. Synthesis of thioglycosides **14**–**17**.

3. Pharmacology

3.1. Materials and methods

Potential cytotoxicity effect of the newly synthesized compounds in four concentrations, were evaluated in the National Institute of Cancer, Cairo Egypt by SRB assay [31]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of plate. Different concentrations of each compound under test (0, 1, 2.5, 5 and 10 μg/mL) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulfo-rhodamine B strain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Coor intensity was measured in an ELISA reader. Finally, the relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.



Scheme 4. Synthesis of pyrazolo (triazole and/or triazine) thioglycosides **21** and **22**.

3.2. Anticancer screening studies

Four of the newly synthesized compounds were screened for their anticancer activities, the two cell lines one dose assay has been done for compounds **8**, **14**, **16** and **22**. The 2-cell lines used in the present investigation are MCF-7 (breast) and HEPG2 (liver). IC_{50} was calculated with regard to saline control group and potency was calculated with regard to percentage of change of tamoxifen or 5-flurouracil and tested compounds, as depicted in Table 1.

Our SAR study shows that all the tested compounds have high or moderate anti-tumor activity towards both the two cell lines with IC_{50} values ranging from 2.67–20.25 (μg/mL) for breast cell line (MCF-7) and 4.62–43.6 (μg/mL) for Liver cell line (HEPG2) compared with both standard drugs. Oxadiazoles carrying two substituents (**14** and **16**) especially when contain thioglycosidic linkage at C2 are more active than those carrying one substituent (compound **8**). Compound **16** was highly specific and potent for both the two cell lines (MCF-7 and HEPG2). The substituents at C2 and C5 of 1, 3, 4-oxadiazole ring shown to be vital for potency. Suggesting specific interaction of these groups with biological targets. Compound **22** has very excellent and specific anti-tumor activity for HEPG2 this may be attributed to the presence of pyrimidine skeleton similar to 5-flurouracil Fig. 1.

4. Toxicity studies

4.1. Animals and treatment

All the compounds tested for their anticancer activity **8**, **14**, **16** and **22** showed very good or moderate anticancer activity in terms of growth inhibitory effect on cancer cell lines. Hence, it could be a potential drug candidate for cancer treatment. Hence these particular compounds were analyzed for its acute toxicity (lethal dose) which is one of the basic requirements in fixing the therapeutic dose in drug development. Male albino rats of the Wistar strain (*Rattus norvegicus*) weighing 110–115 g were obtained from Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt. Animals were housed in clean plastic cages in the laboratory animal room (23 ± 2 °C) on the standard pellet diet and tap water *ad-libitum*, a minimum relative humidity of 40% and a 12 h dark/light cycle. Rats were allowed to acclimate to laboratory conditions for 7 days prior to dosing. All compounds were dissolved in DMSO and administered by gavages at a fixed volume of 0.5 mL/rats. Animals were randomly divided into five groups, eight rats of each. Four groups were used for single dose treatments of compounds **8**, **14**, **16**, and **22** at 500 mg/kg body weight, and one served as control (0.5 mL DMSO/rat). Then mortality of treated-rats was recorded after 24 h. The experimental protocols and procedures were approved by the Local Ethics Committee at the National Research Centre (NRC), Dokki, Cairo, Egypt, and confirmed with the Guide for the Care and Use of Laboratory Animals (NRC, 1999).

Table 1
Cytotoxcity of the synthesized candidates on breast (MCF-7) and liver (HEPG2) cancer cell lines.

Compd. No.	IC_{50} (μg/mL) Breast cell line (MCF-7)	IC_{50} (μg/mL) Liver cell line (HEPG2)
8	17.9	43.6
14	7.1	13.9
16	2.67	4.62
22	20.25	9.1
(TAM)[a]	8.31	–
(5FU)[b]	–	25

[a] TAM (tamoxifen), standard drug for breast cancer.
[b] 5FU (5-flurouracil), standard drug for liver cancer.

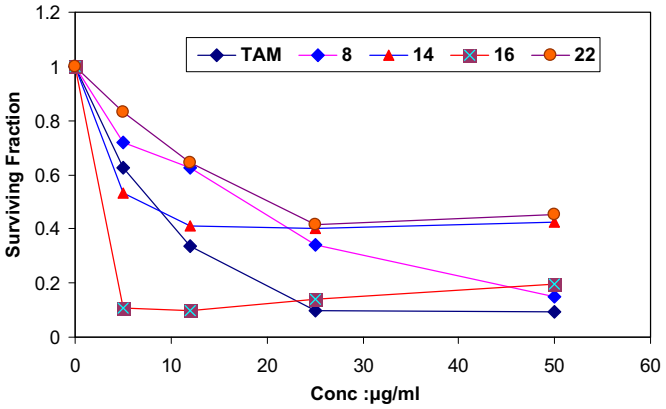


Fig. 1. Representative Graph showing survival of MCF-7 cell grown compared to Tamoxifen (TAM) in the presence of increasing concentrations of compounds **8**, **14**, **16** and **22**.

4.2. Acute oral toxicity

Our results in the present study showed that no mortality in rats traded with compound **8** while compounds **14**, **16** and **22** caused 25%, 25% and 37.50% mortality at 500 mg/kg b.wt, respectively. These results indicated that the lethal doses (LD_{50}) of tested compounds are >500 mg/kg b.wt and all compounds are considered a moderate toxicity based on oral LD_{50} values (no label, >500 <2000 mg/kg), which recommended by the Organization for Economic Co-operation and Development [30] Fig. 2.

5. Conclusion

We have achieved the synthesis of *1H*-pyrazolo-1,3,4-oxadiazole derivatives having cyclic and acyclic carbohydrate residues through S-glycosidic bond formation in an efficient manner. Pharmacological evaluation of compounds **8**, **14**, **16** and **22** against 2-cell lines MCF-7 (breast) and HEPG2 (liver) revealed them to possess high or moderate anti-tumor activities. Compounds **14**, **16** and **22** showed very good anticancer activity in terms of growth inhibitory effect on both cancer cell lines. Hence, it could be a potential drug candidate for cancer treatment. None of the tested compounds presented any toxicity in doses ranging from 50 to 500 mg kg^{-1} of

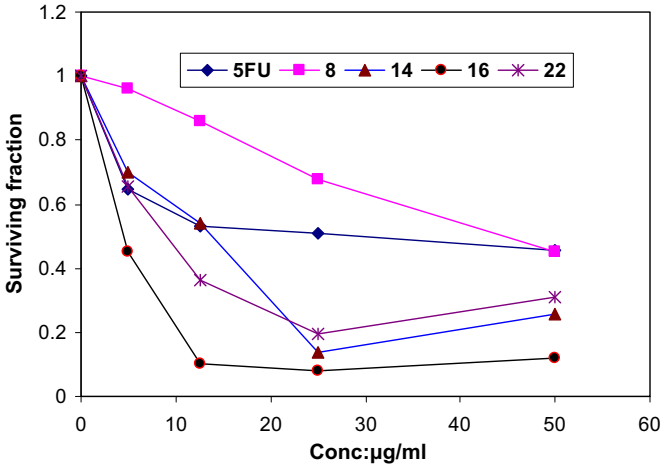


Fig. 2. Representative Graph showing survival of HEPG2 cell grown compared to 5-Flurouracil (5FU) in the presence of increasing concentrations of compounds **8**, **14**, **16** and **22**.

the animal body weight. The lethal doses (LD_{50}) of tested compounds are >500 mg/kg b.wt.

6. Experimental

All melting points were determined on an Electrothermal 9100 digital melting point apparatus. NMR and mass spectra were recorded on: ^1H, ^{13}C NMR spectra were determined using Jeol JMS-AX 500 MHz and TMS as internal standard. Chemical shifts are expressed as δ (ppm). Mass spectra were recorded on Varian MAT 311 A at 70 ev. Elemental analyses were carried out at the micro-analytical unit, Faculty of Science, Cairo University. Precoated silica gel 60 F_{254}plats with a layer thickness 0.25 nm from Merck were used for thin layer chromatography. Yields are not optimized.

6.1. General procedure for synthesizing of **2a** and **2b**

To 2-butanone or methyl *iso*-butyl ketone (10 mmol) in acetic acid (100 mL) and water (10 mL) was added phenylhydrazine (11 mmol). The reaction mixture was stirred at room temperature until completion (TLC, 2 h). The reaction mixture was diluted with an additional 50 mL of water and subsequently extracted with ethyl acetate (3 × 50 mL). The organic layer was separated, washed with water, dried and evaporated under reduced pressure to give:

6.1.1. 1-(Butan-2-ylidene)-2-phenylhydrazine (**2a**)

As viscous yellow oil, yield 78%; ^1H NMR ($CDCl_3$): δ 0.96 (t, 3H, CH_3), 1.75 (q, 2H, CH_2), 2.35 (s, 3H, CH_3), 6.90–7.35 (m, 6H, Ph–H, NH). MS, *m*/*z* (%): 162 [M^+] (90), 147.6 (5), 133 (4), 85.2 (12), 77 (86). Anal. Calcd. For. $C_{10}H_{14}N_2$ (162.28): C, 74.03; H, 8.70; N, 17.27. Found: C, 74.22; H, 8.51; N, 17.40.

6.1.2. 1-(4-Methylpenten-2-ylidene)-2-phenylhydrazine (**2b**)

As yellow crystals (ethanol), yield 75%; m.p. 131–132 °C; ^1H NMR ($CDCl_3$): δ 0.86, 0.88 (2s, 6H, 2× CH_3), 1.96 (m, 1H, CH), 2.04 (s, 3H, CH_3), 2.97 (d, 2H, J = 6.90 Hz, CH_2), 6.98–7.65 (m, 5H, Ph–H), 7.91(s, 1H, NH). MS, *m*/*z* (%): 190 [M^+] (100), 175.9 (8), 148.8 (5), 134.9 (4), 97(4), 76.7 (86). Anal. Calcd. For. $C_{12}H_{18}N_2$ (190.28): C, 75.74; H, 9.53; N, 14.72. Found: C, 75.90; H, 9.41; N, 14.55.

6.2. General procedure for synthesizing of **3** and **4**

Phosphorous oxychloride (25 mmol) was added to DMF (100 mL) at 0 °C and stirred for 30 min **2b** or **2a** (10 mmol) were added slowly to this mixture and stirred for 5 h. The crude reaction was then quenched into water (1 L) and stirred for an additional 1 h and extracted with ethyl acetate (3 × 50 mL). The organic layer was separated, washed with water, dried and evaporated under reduced pressure. The crude was purified using column chromatography (pet.ether/ethyl acetate 8:2, R_f = 0.35) to afford:

6.2.1. 3-Isobutyl-1-phenyl-1H-pyrazole-4-carbaldehyde (**3**)

Colorless crystals (ethanol); yield 80%; m.p. 168–170 °C. IR (KBr, cm^{-1}) υ 2950, 2877 (CH), 1682 (CO), 1594 (C=C); ^1H NMR (DMSO-d_6): δ 0.86, 0.88(2s, 6H, 2× CH_3), 1.96 (m, 1H, CH), 2.71 (d, 2H, J = 6.90 Hz, CH_2), 7.43–7.83 (m, 5H, Ph–H), 9.07 (s, 1H, pyrazole H-5), 9.89 (s, 1H, CHO). ^{13}C NMR: δ 22.54 (2C, CH_3), 28.82(CH), 36.12 (CH_2), 119.71 (2C, Ar–C), 123.15 (pyrazole C4), 127.68 (Ph–C), 129.69 (2C, Ar–C), 131.71(pyrazole C5), 139.21 (Ar–C), 155.48 (pyrazole C2), 184.48 (CHO). MS, *m*/*z* (%): 228 [M^+] (50), 213.2 (30), 199 (3), 183 (63), 157.3 (100), 142.7 (20), 93(73), 77(6). Anal. Calcd. For. $C_{14}H_{16}N_2O$ (228.29): C, 73.66; H, 7.06; N, 12.27. Found: C, 73.50; H, 7.22; N, 12.40.

6.2.2. 3, 4-Dimethyl-1-phenyl-1H-pyrazole (**4**)

Colorless solid (ethanol); yield 76%; m.p. 86–88 °C ^1H NMR (DMSO-d_6): δ 1.94 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 7.11–7.42 (m, 5H, Ph–H), 8.09 (s, 1H, pyrazole H-5). Anal. Calcd. For. $C_{11}H_{12}N_2$ (172.23): C, 76.71; H, 7.02; N, 16.27. Found: C, 76.90; H, 7.20; N, 16.35.

6.3. 3-Isobutyl-1-phenyl-1H-pyrazole-4-carboxylic acid (**5**)

Compound **3** (10 mmol) was dissolved in 20% sulphuric acid (10 mL) then, aqueous solution of 30% $K_2Cr_2O_7$ (20 mL) was added slowly within 30 min. The reaction mixture was refluxed for another 1 h, allowed to cool to room temperature and poured onto ice water. A solid product precipitate was filtered off, washed with water (20 mL) dried and crystallized from ethanol to give **5** as colorless crystals. Yield 82%, m.p. 158–159 °C; IR (KBr, cm^{-1}) υ 3046–3399 (OH), 2954, 2869(CH), 1696(CO), 1600(C=N); ^1H NMR (DMSO-d_6): δ 0.88, 0.90 (2s, 6H, 2× CH_3), 2.01 (m, 1H, CH), 2.70 (d, 2H, J = 6.9 Hz, CH_2), 7.30–7.85 (m, 5H, Ph–H), 8.84 (s, 1H, pyrazole H-5), 12.82 (brs., 1H, OH). MS, *m*/*z* (%): 244 [M^+] (50), 201.8 (100), 158 (25), 142.2 (9), 77 (4). Anal. Calcd. For. $C_{14}H_{16}N_2O_2$ (244.29): C, 68.83; H, 6.60; N, 11.47. Found: C, 68.70; H, 6.81; N, 11.35.

6.4. Ethyl-3-isobutyl-1-phenyl-1H-pyrazole-4-carboxylate (**6**)

Compound **5** (10 mmol) in absolute ethanol (20 mL) contains sulphuric acid (5 drops) was refluxed for 2 h. the reaction mixture was then cooled to room temperature poured onto ice water, neutralized to pH 6–7 by adding $NaHCO_3$ in small potions and extracted using ethyl acetate (50 mL). The two phases were separated and aqueous phase was extracted with ethyl acetate (3 × 10 mL), the organic phase was dried over anhydrous sodium sulphate then evaporated under reduced pressure and the residue was crystallized from ethanol to give **6** as a white solid. Yield 72%; m.p. 49–50 °C; IR (KBr, cm^{-1}) υ 2959, 2868 (CH), 1698 (COOEt), 1597 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 0.98, 0.99 (2s, 6H, 2× CH_3), 1.36 (t, 3H, CH_3-ester), 2.11 (m, 1H, CH), 2.82 (d, 2H, J = 6.85 Hz, CH_2), 4.31 (q, 2H, CH_2 _ester), 7.45–7.69 (m, 5H, Ph–H), 8.34 (s, 1H, pyrazole H-5). Anal. Calcd. For. $C_{16}H_{20}N_2O_2$ (272.34): C, 70.56; H, 7.40; N, 10.29. Found: C, 70.71; H, 7.22; N, 10.40.

6.5. 3-Isobutyl-1-phenyl-1H-pyrazole-4-carbohydrazide (**7**)

Compound **6** (10 mmol) and hydrazine hydrate (10 mmol) in 250 mL round-bottomed flask was refluxed in ethanol (20 mL) in the presence of catalytic amount of triethyl amine for 8 h. The reaction mixture was allowed to cool to room temperature and poured onto ice water. The resulting brown solid was filtered off and crystallized from ethanol to afford **7**as yellow crystals. Yield 80%; m.p. 270–272 °C; IR (KBr, cm^{-1}) υ 3409, 3320 (NH_2), 3135 (NH), 2969, 2878 (CH), 1668 (CO), 1597 (C=C); ^1H NMR (500 MHz, DMSO-d_6): δ 0.86, 0.88 (2s, 6H, 2× CH_3), 2.01 (m, 1H, CH), 2.73 (d, 2H, J = 6.8 Hz, CH_2), 4.20 (s, 2H, NH_2), 7.47–7.70 (m, 5H, Ph–H), 8.73 (s, 1H, pyrazole H-5), 9.18 (s, 1H, NH). Anal. Calcd. For. $C_{14}H_{18}N_4O$ (258.32): C, 65.09; H, 7.02; N, 21.69. Found: C, 65.20; H, 7.11; N, 21.80.

6.6. 5-(3-Isobutyl-1-phenyl-1H-pyrazole-4-yl)-1,3,4-oxadiazle-2-thiol (**8**)

To a solution of compound **7** (10 mmol) in ethanolic potassium hydroxide (20 mL), carbon disulphide (2 mL) was dropped in over period of half hour at room temperature (25 °C). After an additional half hour of stirring, a precipitate was formed and collected by filtration. The salt formed was dissolved in water, then acidify with

HCl the precipitate thus formed was filtered off and crystallized from methanol to give **8** as pale yellow solid. Yield 72%, m.p. 160–161 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91, 0.92 (2s, 6H, 2× CH_3), 2.01 (m, 1H, CH), 2.73 (d, 2H, J = 6.90 Hz, CH_2) 7.33–7.90 (m, 5H, Ph–H), 9.15 (s, 1H, pyrazole H-5), 14.50 (brs, 1H, SH). Anal. Calcd. For. $C_{15}H_{16}N_4OS$ (300.38): C, 59.98; H, 5.37; N, 18.65. Found: C, 59.80; H, 5.50; N, 18.70.

6.7. General procedure for synthesizing of **14–17**

To a solution of **8** (10 mmol) in dry DMF (20 mL), NaH (15 mmol) was added portionwise through 15 min and the solution stirred at room temperature for another 30 min. Then, a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco (or galacto)pyronosyl bromide **9** or **10**, or chloromethyl methylthioether (**11**), or chloromethyl ethyl ether (**12**) in DMF (10 mL) was dropped within 30 min and the reaction mixture was stirred at room temperature until completion (TLC, 3–6 h). After completion, the reaction mixture was poured on water and acidified with diluted acetic acid. The aqueous phase was extracted with ethyl acetate (3 × 20 mL), the combined organic phase was washed with water dried over anhydrous sodium sulphate. Removal of solvent gave a residue, which was purified by column chromatography using an appropriate solvent system to give compounds **14–17**.

6.7.1. 5-(3-Iso-butyl-1-phenyl-1H-pyrazol-4-yl)-2-(2′,3′,4′,6′-tetra-O-aceyl-β-D-glucopyranosylthio)-1,3,4-oxadiazole (**14**)

White solid; yield 69%, m.p. 95–96 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91, 0.92 (2s, 6H, 2× CH_3), 1.84–1.93 (4s, 12H, 4× OAc), 1.96 (m, 1H, CH), 2.81 (d, 2H, J = 6.85 Hz, CH_2), 4.01 (dd, 1H, J = 2.2, 12.2 Hz, 6′-H), 4.09 (m, 2H, 5′-H, 6′-H), 4.97 (t, 1H, J = 9.5 Hz, 4′-H), 5.13 (t, 1H, J = 9.3 Hz, 2′-H), 5.66 (t, 1H, J = 9.5 Hz, 3′-H), 5.68 (d, 1H, $J_{1',2'}$ = 9.95 Hz, 1′-H), 7.3–7.89 (m, 5H, Ph–H), 9.11 (s, 1H, pyrazole H-5). ^{13}C NMR: δ 20.78–20.84 ($4CH_3$·CO), 22.79 (CH_3), 22.82 (CH_3), 28.10 (CH), 36.20(CH_2), 62.21 (C′6), 68.19 (C′4), 70.31 (C′2), 73.14 (C′3), 75.54 (C′5), 82.53 (C′1), 106.79 (pyrazole C4), 119.28 (2C, Ar–C), 127.66 (Ar–C), 129.97(2C, Ar–C), 130.19 (pyrazole C5), 139.28 (Ar–C), 152.52 (pyrazole C3), 159.43 (oxadiazole C5), 162.21 (oxadiazole C2), 169.76–170.07 ($4COCH_3$). MS, m/z (%): 630 [M^+] (50), 285 (22), 258 (100), 228 (82), 77 (3). Anal. Calcd. For. $C_{29}H_{34}N_4O_{10}S$ (630.67): C, 55.23; H, 5.43; N, 8.88. Found: C, 55.40; H, 5.20; N, 8.70.

6.7.2. 5-(3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)-2-(2′,3′,4′,6′-tetra-O-aceyl-β-D-galactopyranosylthio)-1,3,4-oxadiazole (**15**)

White solid; yield 65%, m.p. 96–98 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.90, 0.92 (2s, 6H, 2× CH_3), 1.84–1.93 (4s, 12H, 4× OAc), 1.96 (m, 1H, CH), 2.81 (d, 2H, J = 6.85 Hz, CH_2), 4.20 (m, 2H, 6′, 6′′-H), 4.37 (m, 1H, 5′-H), 5.12 (t, 1H, J = 9.5 Hz, 4′-H), 5.25 (t, 1H, J = 9.3 Hz, 3′-H), 5.66 (t, 1H, J = 9.5 Hz, 2′-H), 6.24 (d, 1H, $J_{1',2'}$ = 10.39 Hz, 1′-H), 7.31–7.89 (m,5H, Ph–H), 9.11 (s,1H, pyrazole H-5); ^{13}C NMR: δ 19.98–20.80 (4 CH_3 CO), 22.79 (CH_3), 22.88 (CH_3), 28.25 (CH), 36.15(CH_2), 61.90 (C′6), 68.20(C′4), 68.95 (C′2), 72.25 (C′3), 75.20 (C′5), 81.55(C′1), 105.79 (pyrazole C4), 119.28 (2C, Ar–C), 127.66 (Ar–C), 129.97(2C, Ar–C), 139.28 (Ar–C),130.55 (pyrazole C5), 152.58 (pyrazole C3), 158.66 (oxadiazole C5), 161.55 (oxadiazole C2), 169.70–170.15 ($4COCH_3$). Anal. Calcd. For. $C_{29}H_{34}N_4O_{10}S$ (630.67): C, 55.23; H, 5.43; N, 8.88. Found: C, 55.30; H, 5.32; N, 8.90.

6.7.3. 5-(3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)-2-[((methylthio) methyl)thio]-1,3,4-oxadiazole (**16**)

White solid; yield 66%, m.p. 50–52 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.87, 088 (2s, 6H, 2× CH_3), 1.97 (m, 1H, CH), 2.11 (s, 3H, CH_3), 2.70 (d, 2H, J = 6.9 Hz, CH_2), 3.77 (s, 2H, CH_2), 7.29–7.86 (m, 5H, Ph–H), 8.89 (s, 1H, pyrazole H-5); ^{13}C NMR: δ 14.47 (CH_3), 22.58 (2C, $2CH_3$), 28.56 (CH), 36.46 (CH_2), 60.12 (CH_2), 114.23 (pyrazole C4), 119.52 (2C, Ar–C), 127.15 (Ar–C), 129.57 (2C, Ar–C), 131.34 (pyrazole C5), 139.52 (Ar–C), 151.22 (pyrazole C3), 155.66 (oxadiazole C5), 163.58 (oxadiazole C2). Anal. Calcd. For. $C_{17}H_{20}N_4OS_2$ (360.50): C, 56.64; H, 5.59; N, 15.54. Found: C, 56.80; H, 5.40; N, 15.60.

6.7.4. 5-(Ethoxymethylthio)-2-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (**17**)

White solid; yield 77%, m.p. 112–114 °C; IR (KBr, cm^{-1}) υ 3060, 2920, (CH), 1600 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) 0.88, 0.89 (2s, 6H, 2× CH_3), 2.01 (m, 1H, CH), 2.60 (s, 3H, CH_3), 2.66–2.73 (m, 4H, 2× CH_2), 4.20 (s, 2H, CH_2), 7.25–7.88 (m, 5H, Ph–H), 8.90 (s, 1H, H-5 pyrazole). Anal. Calcd. For. $C_{18}H_{22}N_4O_2S$ (358.46): C, 60.31; H, 6.19; N, 15.63. Found: C, 60.40; H, 6.10; N, 15.50.

6.8. General procedure for synthesizing of **19** and **20**

Compound **3** (10 mmol) was refluxed with an equimolecular amount of **18a** or **18b** in absolute ethanol (30 mL) in the presence of piperidine (4 drops) for 10 h. A precipitate was formed on hot was filtered off and recrystallized from acetic acid to give **19** and **20** as a pale yellow solid, respectively.

6.8.1. 4-[((3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)methylene) amino]-4H-1,2,4-triazole-3-thiol (**19**)

Yield 77%; m.p. 237–238 °C; IR (KBr, cm^{-1}) υ 2959, 2868 (CH), 3106(SH), 2950, 2867(CH), 1598(C=N); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.94 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 2.1 (m, 1H, CH), 2.77 (m, 2H, CH_2), 7.44–7.68 (m, 5H, Ph–H), 7.97 (s, 1H, CH=N), 8.35 (s, 1H, triazole CH=N), 8.37 (s, 1H, pyrazole H-5), 10.23 (s, 1H, SH). Anal. Calcd. For. $C_{16}H_{18}N_6S$ (326.42): C, 58.87; H, 5.56; N, 25.75. Found: C, 58.70; H, 5.71; N, 25.60.

6.8.2. 4-[((3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)methylene) amino]-3-mercapto-6-methyl-1,2,4-triazin-5(4H)-one (**20**)

Yield 82%; m.p. 188–189 °C; IR (KBr, cm^{-1}) υ 3108(SH), 2958, 2865 (CH), 1698(CO), 1599(C=N); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.93 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 2.01 (s,1H, CH), 2.33 (s, 3H, CH_3), 2.79 (d, 2H, J = 6.85 Hz, CH_2), 7.43–7.90 (m, 6H, Ph–H, SH), 8.22 (s, 1H, CH=N), 8.50 (s, 1H, pyrazole H-5). Anal. Calcd. For. $C_{18}H_{20}N_6OS$ (368.46): C, 58.68; H, 5.47; N, 22.81. Found: C, 58.50; H, 5.58; N, 22.70.

6.9. General procedure for synthesizing of **21** and **22**

To a solution of **19** or **20** (10 mmol) in dry DMF (20 mL), NaH (15 mmol) was added portionwise through 15 min and the solution stirred at room temperature for another 30 min. Then, a solution of 2′,3′,4′,6′-tetra-*O*-acetyl-α-D-glucopyronosyl bromide (**9**) in DMF (10 mL) was dropped within 30 min and the reaction mixture was stirred at room temperature until completion (TLC, 6–8 h). After completion, the reaction mixture was poured on water and acidified with diluted acetic acid. The aqueous phase was extracted with ethyl acetate (3 × 20 mL), the combined organic phase was washed with water dried over anhydrous sodium sulphate. Removal of solvent gave a residue, which was purified by column chromatography using an appropriate solvent system to give compounds **21** and **22**, respectively.

6.9.1. N-[(3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)methylene]-3-(2′,3′,4′,6′-tetra-O-aceyl-β-D-glucopyranosylthio)-4H-1,2,4-triazol-4-amine (**21**)

Compound **21** was purified by column chromatography (pet.ether/ethylacetate 8:2, R_f = 0.45). Yield 75%, m.p. 190–192 °C;

^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.90–1.94 (4s, 12H, 4× OAc), 1.95 (m, 1H, CH), 2.76 (d, 2H, J = 6.8 Hz, CH_2), 3.90 (m, 1H, 5′-H), 4.10 (m, 2H, 6′, 6′-H), 4.92 (t, 1H, J = 9.60 Hz, 4′-H), 5.06 (t, 1H, J = 9.20 Hz, 2′-H), 5.42 (t, 1H, J = 9.6 Hz, 3′-H), 5.73 (d, 1H, $J_{1',2'}$ = 9.75 Hz, 1′-H), 7.47–7.86 (m, 5H, Ph–H), 8.87 (s, 1H, CH═N), 8.97(s, 1H, triazole CH═N), 9.32 (s, 1H, pyrazole H-5). Anal. Calcd. For. $C_{30}H_{36}N_6O_9S$ (656.71): C, 54.87; H, 5.53; N, 12.80. Found: C, 54.70; H, 5.60; N, 12.70.

*6.9.2. 4-[((3-Isobutyl-1-phenyl-1H-pyrazol-4-yl)methylene) amino]-6-methyl-3-(2′,3′,4′,6′-tetra-O-aceyl-β-D-glucopyranosylthio)-1,2,4-triazin-5(4H)-one (**22**)*

Compound **22** was purified by using column chromatography (pet.ether/ethylacetate 6:1, R_f = 0.35). Yield 78%, m.p. 140–142 °C; IR (KBr, cm^{-1}) υ 2958, 2866(CH), 1752(OCOCH$_3$), 1698(CO), 1602 (C═N); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 2.00–2.08 (m, 13H, 4× OAc, CH), 2.33 (s, 3H, CH_3), 2.73 (d, 2H, J = 6.90 Hz, CH_2), 3.92 (m, 1H, 5′-H), 4.11 (dd, 1H, J = 2.30, 12.30 Hz, 6′-H), 4.24 (m, 1H, 6′′-H), 5.20 (t, 1H, J = 9.60 Hz, 4′-H), 5.35 (t, 1H, J = 9.3 Hz, 2′-H), 5.70 (t, 1H, J = 9.50 Hz, 3′-H), 6.81 (d, 1H, $J_{1,2}$ = 9.20 Hz, 1′-H), 7.43–7.68 (m, 5H, Ph–H), 8.23 (s, 1H, CH═N), 8.44 (s, 1H, pyrazole H-5). ^{13}C NMR: δ 17.17 (CH_3), 20.66–20.82 (4CH_3CO), 22.62 (2C, 2CH_3), 28.80 (CH), 36.21(CH_2), 61.72 (C′6), 67.93 (C′4), 68.98 (C′2), 73.96 (C′3), 74.55 (C′5), 86.78 (C′1), 115.32 (pyrazole C4), 119.62 (2C, Ar–C), 127.48 (Ar–C), 129.40 (2C, Ar–C), 129.65 (pyrazole C5), 139.30 (Ar–C), 146.55, 148.92, 155.35, 164.85, 169.07–170.21 (4 $COCH_3$), 170.66 (CO). MS, m/z (%): 698 [M$^+$] (1), 212 (40), 183 (100), 77 (14). Anal. Calcd. For. $C_{32}H_{38}N_6O_{10}S$ (698.74): C, 55.00; H, 5.48; N, 12.03. Found: C, 55.10; H, 5.60; N, 12.20.

References

[1] J. Li, L.Z. Xu, K.L. He, W.J. Juo, Y.H. Zheng, P. Xia, Y. Chen, Breast Cancer Res. 3 (2001) 253–263.
[2] J. Engel, R. Eckel, J. Kerr, M. Schmidt, G. Furstenberger, R. Richter, H. Sauer, H.J. Senn, Ital. J. Anat. Embryol. 106 (2001) 59–68.
[3] N. Uchiyama-Kokubu, T. Watanabe, Anticancer Drugs 12 (2001) 769–779.
[4] I.A. Cree, L. Knight, F. Di Nicolantonio, S. Sharma, T. Gulliford, Curr. Opin. Investig. Drugs 3 (2002) 634–640.
[5] I.F. Faneyte, P.M.P. Kristel, M.J. van de Vijver, Intl. J. Cancer 93 (2001) 114–122.
[6] P. Brown, D.J. Best, N.J.P. Broom, R. Cassels, P.J. O'Hanlon, T.J. Mitchell, N.F. Osborne, J.M. Wilson, J. Med. Chem. 40 (1997) 2563–2570.
[7] M. Harfenist, D.J. Heuser, C.T. Joyner, J.F. Batchelor, H.L. White, J. Med. Chem. 39 (1996) 1857–1863.
[8] C. Chen, C.H. Senanayake, T.J. Bill, R.D. Larsen, T.R. Verhoeven, P.J. Reider, J. Org. Chem. 59 (1994) 3738–3741.
[9] M. Amir, K. Shikha, Eur. J. Med. Chem. 39 (2004) 535–545.
[10] S. Vardan, H. Smulyan, S. Mookherjee, R. Eich, Clin. Pharmacol. Ther. 34 (1983) 290–296.
[11] H.L. Yale, K. Losee, J. Med. Chem. 9 (1966) 478–483.
[12] C.R.W. Guimaraes, D.L. Boger, W.L. Jorgensen, J. Am. Chem. Soc. 127 (2005) 1737–1743.
[13] D. Han, X.B. Meng, L.N. Wang, H. Liu, Y. Yao, Z. Wang, Z.J. Yang, Z.M. Liu, Z.J. Li, Tetrahedron Asymmetry 20 (2009) 399–410.
[14] L. Mishra, M.K. Said, H. Itokawa, K. Takeya, Bioorg. Med. Chem. 3 (1995) 1241–1245.
[15] D. Kumar, S. Sundaree, E.O. Johnson, K. Shah, Bioorg. Med. Chem. Lett. 19 (2009) 4492–4494.
[16] W.S. Li, S.V. More, C.H. Wang, Y.C. Jen, C.F. Yao, T.F. Wang, C.C. Hung, S.C. Jao, Bioorg. Med. Chem. Lett. 20 (2010) 1148–1152.
[17] P.G. Baraldi, A. Bovero, F. Fruttarolo, R. Romagnoli, M.A. Tabrizi, D. Preti, K. Varani, P.A. Borea, A.R. Moorman, Bioorg. Med. Chem. 11 (2003) 4161–4169.
[18] M.C. Cardia, L. Corda, A.M. Fadda, A.M. Maccioni, E. Maccioni, A. Plumitallo, ILFarmaco 53 (1998) 698–708.
[19] A.M. Farag, A.S. Mayhoub, S.E. Barakat, A.H. Bayomi, Bioorg. Med. Chem. 16 (2008) 881–889.
[20] Y. Xia, C.D. Fan, B.X. Zhao, J. Zhao, D.S. Shin, J.Y. Miao, Eur. J. Med. Chem. 43 (2008) 2347–2353.
[21] F. Bozzo, A. Bassignana, L. Lazzarato, D. Boschi, A. Gasco, C. Bocca, A. Miglietta, Chem. Biol. Interact. 182 (2009) 183–190.
[22] G.H. Elgemeie, M.M. Hussein, S.A. Al-Khursani, J. Carbohydr. Chem. 23 (2004) 465–481.
[23] G.H. Elgemeie, W.A. Zaghary, K.M. Amin, T.M. Nasr, J. Carbohydr. Chem. 27 (2008) 373–378.
[24] G.H. Elgemeie, W.A. Zaghary, K.M. Amin, T.M. Nasr, J. Carbohydr. Chem. 28 (2009) 161–178.
[25] G.H. Elgemeie, E.H. Eltammy, I.I. Elgawad, N.M. Mahmoud, Synth. Commun. 39 (2009) 443–458.
[26] H.N. Hafez, A.B. El-Gazzar, G.A.M. Nawwar, Eur. J. Med. Chem. 45 (2010) 1485–1493.
[27] E.S.M. Yakout, Y.A. Allam, G.A.M. Nawwar, Heteroatom Chem. 10 (1999) 177–182.
[28] A.M. Attia, G.H. Elgemeie, Synth. Commun. 33 (2003) 2243–2255.
[29] I.W. Still, N. Plavac, T.M. Mackinnon, M.S. Chauban, Can. J. Chem. 54 (1976) 280–289.
[30] L. Stefaniak, Org. Magn. Reson. 12 (1979) 379–382.
[31] P. Skehan, R. Strong, D. Scadiaro, A. Monks, J. Mc-Mahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyed, J. Natl. Cancer Inst. 82 (1990) 1107–1112.