Journal of Sciences, Islamic Republic of Iran 26(4): 321 - 325 (2015) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Synthesis and Evaluation of Ethyl 2,4-dioxo-4-arylbutanoate Derivatives as Src Kinase Inhibitors

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Received: 22 July 2015 / Revised: 10 August 2015 / Accepted: 26 September 2015

Abstract

In this work, different ethyl 2,4-dioxo-4-arylbutanoate derivatives were prepared and evaluated for their Src Kinase inhibitory activity. For this purpose, the appropriate substituted acetophenone derivatives reacted with diethyl oxalate in the presence of sodium ethoxide in dried ethanol to give the corresponding products. All compounds showed moderate activities comparing with staurosporine as the reference drug.

Keywords: Ethyl 2,4-dioxo-4-arylbutanoates; Src kinase; Cancer.

Introduction

Src family tyrosine kinases (SFKs) are the subject of major interest due to their critical roles in signal transduction pathways and cellular functions such as invasion, division, differentiation, survival, adhesion and migration [1]. Src also known as c-Src is the first identified proto-oncogene and one of the most widely studied members of this family. There is a significant evidence demonstrating over expression or mutation of Src kinase correlates with tumor growth and metastasis in several human malignancies, including colon, breast, and pancreatic cancers [2]. In recent studies, the interest in Src kinase inhibitors has been increased for the treatment of cancer as an antiinvasion strategy [3-5]. In this regard, various synthetic compounds such as tetrahydroindazolones [6], thiazolyl *N*-benzyl-substituted acetamides [7], cyclic peptides [8], and purine derivatives [9] have been synthesized exhibiting Src inhibitory activities.

1,3-Diketones are significant building blocks [10] in organic synthesis and have shown promising biological activities such as antitumor [11], antimicrobial [12], antibacterial and antiviral [13], and HIV-1 integrase inhibitory [14] activities. Also, some derivatives have depicted activity versus P388 lymphocytic leukemia in mice [15] and the reported diketone based vanadium complexes by Vijayaraghavan et al. were emerged as insulin mimetics for possible diabetes therapy [16]. These findings revealed that 1,3-diketones need special attention in drug discovery developments.

In continuation of our studies for the synthesis of

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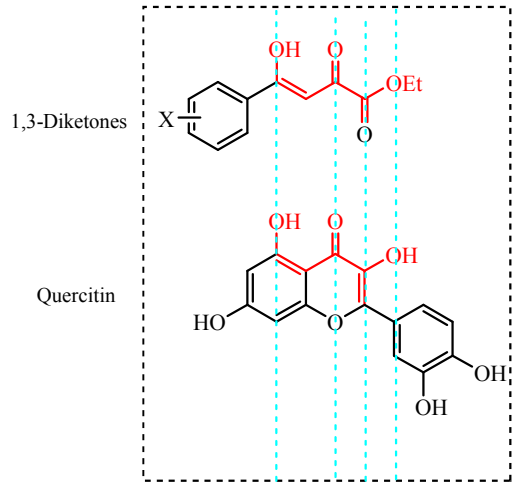


Figure 1. Design of 1,3-Diketones as kinase Inhibitors

bioactive compounds [17-22], we focused on quercetin, a polyol as a model, and in this respect, ethyl 2,4-dioxo-4-phenylbutanoates absorbed our attention to find Src kinase inhibitors (Figure 1). For this purpose, different ethyl 2,4-dioxo-4-arylbutanoates were synthesized and evaluated for their Src kinase inhibitory activities (Scheme 1).

Materials and Methods

All starting materials, reagents, and solvents were purchased from Merck and Aldrich. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer and chemical shifts are expressed as ppm with tetramethylsilane (TMS) as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrophotometer (in KBr).

General procedure for the synthesis of ethyl 2,4-dioxo-4-arylbutanoates 3a-f

A mixture of diethyl oxalate (10 mmol) and appropriate acetophenones (10 mmol) was added drop wise to a stirred solution of freshly prepared NaOEt (10 mmol Na in 10 mL dried ethanol). After stirring overnight the reaction mixture was heated at 80 °C for 30 min. The reaction mixture was acidified with sulfuric acid (pH = 2) and extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under vacuum. All compounds were recrystallized from EtOH to obtain pure products 3a-f.

Ethyl-4-hydroxy-2-oxo-4-phenylbut-3-enoate 3a: Mp: 35-37 °C. IR (KBr): 3082, 2995, 2980, 2935, 1735, 1637, 1520 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.42 (t, J = 7.5 Hz, 3H, CH_3), 4.40 (q, J = 7.5 Hz, 2H, CH_2), 7.09 (s, 1H, CH), 7.50 (t, J = 7.5 Hz, 2H, H3, H5), 7.61 (t, J = 7.5 Hz, 1H, H4), 7.99 (d, J = 7.5 Hz, 2H, H2, H6).

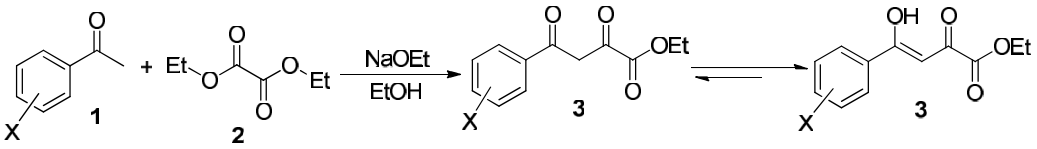
*Ethyl-4-(2-chlorophenyl)-4-hydroxy-2-oxobut-3-enoate **3b***: Mp: 51-53 °C. IR (KBr): 3097, 2998, 2981, 2938, 1739, 1635, 1522 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (t, J = 7.0 Hz,3H, CH_3), 4.39 (q, J = 7.0 Hz, 2H, CH_2), 6.97 (s, 1H, CH), 7.64 (dd, J = 7.5, 2.0 Hz, 1H, H6), 7.45-7.47 (m, 2H, H3, H5), 7.38 (td, J = 7.5, 2.0 Hz, 1H, H4).

Ethyl-4-(2,4-dichlorophenyl)-4-hydroxy-2-oxobut-3-enoate 3c: Mp: 60-62 °C. IR (KBr): 3088, 2992, 2980, 2935, 1730, 1630, 1520 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (t, J = 7.0 Hz, 3H, CH_3), 4.39 (q, J = 7.0 Hz, 2H, CH_2), 6.95 (s, 1H, CH), 7.37 (dd, J = 8.4, 1.6 Hz, 1H, H6), 7.50 (dd, J = 1.6 Hz, 1H, H3), 7.37 (dd, J = 8.4 Hz, 1H, H5).

Ethyl-4-(4-fluorophenyl)-4-hydroxy-2-oxobut-3-enoate 3d: Mp: 46-48 °C. IR (KBr): 3107, 2995, 2971, 2908, 1732, 1600, 1509 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (t, J = 7.2 Hz, 3H, CH_3), 4.39 (q, J = 7.2 Hz, 2H, CH_2), 7.04 (s, 1H, CH), 7.19 (t, J = 8.4, Hz, 2H, H3, H5), 8.04 (dd, J = 8.4, 4.8 Hz, 2H, H2, H6).

Ethyl-4-hydroxy-4-(4-methoxyphenyl)-2-oxobut-3-enoate 3e: Mp: 29-31 °C. IR (KBr): 3079, 2988, 2939, 2846, 1725, 1603, 1516 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.41 (t, J = 7.2 Hz, 3H, CH_3), 4.39 (q, J = 7.2 Hz, 2H, CH_2), 3.90 (s, 3H, OCH_3), 6.98 (d, J = 8.8 Hz, 2H, H3, H5), 7.03 (s, 1H, CH), 7.99 (d, J = 8.8 Hz, 2H, H2, H6).

Ethyl-4-hydroxy-2-oxo-4-(p-tolyl)but-3-enoate 3f: Mp: 37-39 °C. IR (KBr): 3085, 2988, 2975, 2928, 1725, 1635, 1520 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.42 (t, J



Scheme 1. Synthesis of 2,4-dioxo-4-arylbutanoates 3a-f

= 7.2 Hz, 3H, CH_3), 2.44 (s, 3H, CH_3), 4.30 (q, *J* = 7.2 Hz, 2H, CH_2), 7.07 (s, 1H, CH), 7.39-7.41 (m, 2H, H4, H5), 7.79-7.81 (m, 2H, H2, H6).

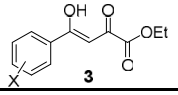
Src Kinase activity assay

The effect of synthesized compounds on the activity of Src kinase was determined using HTScan Src Kinase Assay Kit, catalogue number 7776 from Cell Signaling Technology (Danvers, MA, USA); according to manufacturer's protocol. Streptavidin-coated plates were purchased from Pierce (Rockford, IL, USA) [18]. In brief, the kinase reaction was started with the incubation of the 12.5 µL of the reaction cocktail (0.5 ng/µL of GST-Src kinase in 1.25 mM DTT) with 12.5 µL of prediluted compounds (dissolved in 1% DMSO) for 5 min at room temperature. ATP/substrate (25 µL, 20 µM/1.5 µM) cocktail was added to the mixture. The biotinylated substrate (catalogue number 1366) contains the residues surrounding tyrosine 160 (Tyr 160) of signal transduction protein and has a sequence of EGIYDVP. The reaction mixture was incubated for 30 min at room temperature. The kinase reaction was stopped with the addition of 50 µL of 50mM EDTA (pH 8.0). The reaction solution (25 µL) was transferred into 96-well streptavidin plates (Pierce, part number 15125), diluted with 75 µL double distilled water, and incubated at room temperature for 60 min. At the end of the incubation, the wells were washed three times with 200 µL of 0.05% Tween-20 in PBS buffer (PBS/T). After that 100 µL of phosphotyrosine antibody (P-Tyr-100) (1:1000 dilution in PBS/T with 1% BSA) was added to the each well and the wells were incubated for another 60 min. After washing three times with 0.05% Tween-20 in PBS/T, the wells were incubated with 100 µL secondary anti-mouse IgG antibody, which was HRP-conjugated (1:500 dilution in PBS/T with 1 % BSA) for next 30 min at room temperature. The wells were washed five times with 0.05% Tween-20 in PBS and then were incubated with 100 µL of 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) substrate for 5 min. The reaction was stopped by adding 100 µL /well of stop solution to each well, mixed well, and read the absorbance at 450 nm using a microplate reader (Molecular devices, spectra Max M2). IC_{50} values of the compounds were calculated using ORIGIN 6.0 (origin lab) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All the experiments were carried out in triplicate.

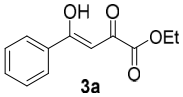
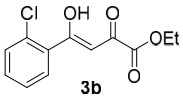
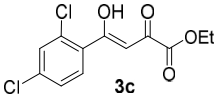
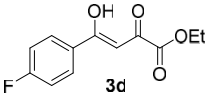
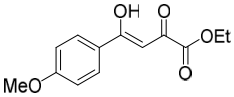
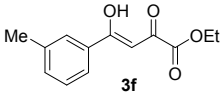
Results and Discussion

2,4-Dioxo-4-arylbutanoate 3a-f were synthesized

Table 1. Src Kinase inhibitory activity of products 3.



3

Entry	X	Product 3	IC_{50} (µM) [a]
1	H	 **3a**	59.2±0.1
2	2-Cl	 **3b**	48.8±0.2
3	2,4-di-Cl	 **3c**	80.4±0.1
4	4-F	 **3d**	90.3±0.3
5	4-OMe		50.4±0.1
6	3-Me	 **3f**	48.3±0.2
7	Staurosporine		0.3±0.1

[a]The concentration of the compound that inhibited enzyme activity by 50%. All the experiments were carried out triplicate.

according to the synthetic routes outlined in Scheme 1. The appropriate substituted acetophenone derivative reacted with diethyl oxalate in the presence of sodium ethoxide (NaOEt) in dried ethanol to give products 3a-f (Table 1).

All the synthesized compounds 3a-f were evaluated for Src kinase inhibitory activity and compared with staurosporine as the reference drug. Based on the calculated IC_{50} values, all products showed moderate activities (48.3-90.3 µM). Our results revealed that compound 3f having 3-methyl substituent on the aromatic ring showed the best activity (IC_{50} = 48.3 µM). Introduction of strong electron-donating group (OMe) to the aromatic ring of product (3e) did not lead to the better activity since Src kinase inhibitory activity (IC_{50} = 50.4 µM) of the related compound was less than compound 3f. However, the presence of 2-chloro substituent on the aromatic ring of synthesized compound (3b) led to the similar activity comparing with 3f. It should be noted that increasing the number chlorine groups (3c) or replacement with fluorine (3d) gave low activity (IC_{50}s = 58.4 and 90.3µM, respectively). Finally, absence of substituents on the aromatic ring of compound 3 exhibited lower activity comparing with 3b, 3e, and 3f possessing 2-chloro, 4-methoxy, and 3-methyl substituents, respectively. Anyway, the activity of compound 3a was higher than compounds 3d and 3f having 2,4-di-chloro and 4-fluoro substituents, respectively.

Conclusion

In conclusion, various ethyl 2,4-dioxo-4-arylbutanoate derivatives were synthesized through the reaction of diethyl oxalate and acetophenones in the presence of sodium ethoxide (NaOEt). Then, products were evaluated for their Src Kinase inhibitory activity. All compounds showed moderate activity comparing with staurosporine as the reference drug.

Acknowledgement

We gratefully acknowledge financial support from the Research Council of Tehran University of Medical Sciences.

References

1. Parsons S.J. and Parsons J.T. Src family kinases, key regulators of signal transduction. *Oncogene* **23**: 7906-7909 (2004).
2. Stehelin D., Fujita D. J. and Padgett T. Detection and enumeration of transformation defective strains of avian sarcoma virus with molecular hybridization. *Virology* **76**:675-684 (1977).
3. Zhang S. and Yu D. Targeting Src family kinases in anti-cancer therapies: turning promise into triumph. *Trends. Pharmacol. Sci*. **33**: 122-128 (2012).
4. Huveldt D., Lewis-Tuffin L.J., Carlson B.L., Schroeder M.A., Rodriguez F., Giannini C., Galanis E., Sarkaria J.N. and Anastasiadis P.Z. Targeting Src Family Kinases Inhibits Bevacizumab-Induced Glioma Cell Invasion. *PLoS One*. **8**: e56505 (2013).
5. Ma J.G., Huang H., Chen S.M., Chen Y., Xin X.L., Lin L.P., Ding J., Liu H. and Meng L.H. PH006, a novel and selective Src kinase inhibitor, suppresses human breast cancer growth and metastasis *in vitro* and *in vivo*. *Breast Cancer Res. Treat*. **130**: 85-96 (2011).
6. Rao V.K., Chhikara B.S., Tiwari R., Shirazi A.N., Parang K. and Kumar A. One-pot regioselective synthesis of tetrahydroindazolones and evaluation of their antiproliferative and Src kinase inhibitory. *Bioor. Med. Chem. Lett*. **22**: 410-414 (2012).
7. Fallah-Tafti A., Foroumadi A., Tiwari R., Shirazi A.N., Hangauer D.G., Bu Y., Akbarzadeh T., Parang K. and Shafiee A. Thiazolyl *N*-benzyl-substituted acetamide derivatives: Synthesis, Src kinase inhibitory and anticancer activities. *Eur. J. Med. Chem*. **46**: 4853-4858 (2011).
8. Shirazi A.N., Tiwari R.K., Brown A., Mandal D., Sun G. and Parang K. Cyclic peptides containing tryptophan and arginine as Src kinase inhibitors. *Bioor. Med. Chem. Lett*. **23**: 3230-3234 (2013).
9. Huang H., Ma J., Shi J., Meng L., Jiang H., Ding J. and Liu H. Discovery of novel purine derivatives with potent and selective inhibitory activity against c-Src tyrosine kinase. *Bioor. Med. Chem*. **18**: 4615-4624 (2010).
10. Nagarapu L., Gaikwad H.K., Sarikonda K., Mateti J., Bantu R., Raghu P.S., Manda K.M. and Kalvendi S.V. Synthesis and cytotoxicity evaluation of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives. *Eur. J. Med. Chem*. **45**: 4720-4725 (2010).
11. Acton N., Brossi A., Newton D.L. and Sporn M.B. Potential prophylactic antitumor activity of retinylidene 1,3-diketones. *J. Med. Chem*. **23**: 805-809(1980).
12. Mulongo G., Mbabazi J., Odongkara B., Twinomuhwezi H. and Mpango G.B. New biologically active compounds from 1,3-diketones. *Res. J.Chem.Sci*. **1**: 102-108 (2011).
13. Sheikh J., Juneja H., Ingle V., Ali P. and Hadda T.B. Synthesis and in vitro biology of Co(II), Ni(II), Cu(II) and Zinc(II) complexes of functionalized beta-diketone bearing energy buried potential antibacterial and antiviral *O*,*O* pharmacophore sites. *J. Saudi Chem. Soc*. **17**: 269-276 (2013).
14. Liming H., Zhipeng L., Zhanyang W., Gengxin L., Xianzhuo H., Xiaoli W. and Chengchu Z. Design, synthesis and biological activity of aromatic diketone derivatives as HIV-1 integrase inhibitors. *Med. Chem*. **11**: 180-187 (2015).
15. Dimmock J.R., Raghavan S.K. and Bigam G.E.

Evaluation of Mannich bases of 2-arylidene-1,3-diketones versus murine P388 leukemia. *Eur. J. Med. Chem*. **23**: 111-117 (1988).

16. Sheela A., Roopan S.M. and Vijayaraghavan R. New diketone based vanadium complexes as insulin mimetics. *Eur. J. Med. Chem*. **43**: 2206-2210 (2008).
17. Fallah-Tafti A., Tiwari R., Shirazi A.N., Akbarzadeh T., Mandal D., Shafiee A., Parang K., Foroumadi A. 4-Aryl-4*H*-chromene-3-carbonitrile derivatives: evaluation of Src kinase inhibitory and anticancer activities. *Med. Chem*. **7**: 466-472 (2011).
18. Rafinejad A., Fallah-Tafti A., Tiwari R., Shirazi A.N., Mandal D., Shafiee A., Parang K., Foroumadi A. and Akbarzadeh T. 4-Aryl-4*H*-naphthopyrans derivatives: one-pot synthesis, evaluation of Src kinase inhibitory and anti-proliferative activities. *Daru J. Pharm. Sci*. **20**: 100 (2012).
19. Mohammadi-Khanaposhtani M., Saeedi M., Zafarghandi N.S., Mahdavi M., Sabourian R., Razkenari E.K., Alinezhad H., Khanavi M., Foroumadi A., Shafiee A. and Akbarzadeh T. Potent acetylcholinesterase inhibitors: design, synthesis, biological evaluation, and docking study of acridone linked to 1,2,3-triazole derivatives. *Eur. J. Med. Chem*. **92**: 799-806 (2015).
20. Rayatzadeh A., Saeedi M., Mahdavi M., Rezaei Z., Sabourian R., Mosslemin M.H., Akbarzadeh T., Foroumadi A. and Shafiee A. Synthesis and evaluation of novel oxoisoindolines derivatives as acetylcholinesterase inhibitors. *Monatsh. Chem*. **146**: 637-643 (2015).
21. Akbarzadeh T., Noushini S., Taban S., Mahdavi M., Khoshneviszadeh M., Saeedi M., Emami S., Eghtedari M., Sarrafi Y., Khoshneviszadeh M., Safavi M., Divsalar K., Moshafi M.H., Asadipour A., Sabourian R., Edraki N., Firouzi O., Miri R., Shafiee A. and Foroumadi A. Synthesis and cytotoxic activity of novel poly-substituted imidazo[2,1-*c*][1,2,4]triazin-6-amines. *Mol. Diversity* **19**: 273-281 (2015).
22. Mohammadi-Khanaposhtani M., Mahdavi M., Saeedi M., Sabourian R., Safavi M., Khanavi M., Foroumadi A., Shafiee A. and AkbarzadehT. Design, synthesis, biological evaluation, and docking study of acetylcholinesterase inhibitors: new acridone-1,2,4-oxadiazole-1,2,3-triazole hybrids. *Chem. Biol. Drug Des*. doi: 10.1111/cbdd.12609